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AGRICULTURAL EXPERIMENT STATION
OF THE COLLEGE OF AGRICULTURE

FUSARIA OF POTATOES

BY C. D. SHERBAKOFF

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FUSARIA OF POTATOES

FUSARIA OF POTATOES[1]

C. D. SHERBAKOFF

INTRODUCTORY

The purpose of this work has been to lay down a basis for the study of the disease known as the fusarial wilt and dry rot of potatoes, *Solanum tuberosum*.

On the basis of previous work done by various mycologists and plant pathologists on Fusaria of potatoes, it was evident, even before the monographic work of Appel and Wollenweber (1910), that the potato is a host of a number of different species of Fusarium. At the same time the descriptions of these organisms were, almost without exception, so confusing, and the organisms as a rule were apparently so variable, that there was no sure way of identifying a Fusarium isolated from potato with a previously described Fusarium even of the same host. Such a state of affairs led Smith and Swingle (1904:50) to make a general statement as follows: " Many specific names have been given to *Fusaria* growing on the potato, and while some of these names may stand for distinct forms, this is not at all certain. Most of these names undoubtedly are synonyms, and for the purpose of this paper all are regarded as such."

On the other hand, Lindau (1908) and others continued to believe firmly that there were different Fusaria of potatoes, and that *Fusarium oxysporum* as described by Smith and Swingle is nothing else than a " Mischart."

Whether more than one species of Fusarium occurs on this host and, if more than one, on what basis they could be separated, was then the question which in the opinion of the writer needed to be definitely settled before any pathological work with these organisms could be properly done. This involved the accumulation of as many different strains of the Fusaria as was practicable, from all available sources, and their comparison in pure culture on various suitable media and under the same conditions of growth. On this basis the work has been carried on since the autumn of 1911.

---

[1] Presented to the Faculty of the Graduate School of Cornell University as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

When the work was well under way and it became apparent that there are indeed a considerable number of species of Fusarium, it came to the writer's knowledge that the same thing was definitely proved, at least for European conditions, by Appel and Wollenweber (1910) in their *Grundlagen* (page 105), in which they state that most of the Fusaria described by them were isolated from the potato, mainly from the tubers. Since then this work has been continued with still greater confidence in its underlying principles, but now mainly in order to verify the European findings for the American conditions.[2]

All cultures of the Fusaria were obtained by isolations made from diseased parts of the potato plant, mainly from tubers received from pathologists in nearly every experiment station in the United States. Of all the strains of the Fusarium-like organisms thus obtained, over one hundred and twenty organisms at first seemed to differ in some way or another. After a careful comparative study of all these strains, more than half of the number proved to be identical with others.

Some of the remaining organisms were identified with several Fusaria described by Appel and Wollenweber (1910), by Jamieson and Wollenweber (1912), and by Wollenweber (1913, b and c). These are as follows:

Fusarium affine Faut. et Lamb.
F. coeruleum (Lib.) Sacc.
F. dimerum Penz.
F. discolor Ap. et Wr.
F. Martii Ap. et Wr.
F. metacroum Ap. et Wr.
F. oxysporum Schlecht., as described by Wollenweber
F. radicicola Wr.
F. Solani (Mart. p. par.) Ap. et Wr.
F. subulatum Ap. et Wr.
F. trichothecioides Wr.

Still other organisms were found to be very closely related either to some of the species named above or to certain other species, at the same time differing sufficiently from them to be separated on a conservative basis from those species. These organisms were then designated as new varieties of the following old species:

---

[2] The general discussion of the genus Fusarium was known to the writer before he began his work, when in the summer of 1911 Dr. Jensen received the *Grundlagen* and now and then read parts of it to the writer. Having no idea that Appel and Wollenweber were working mainly with the Fusaria of the potato, the writer did not study their work thoroughly as early as he should have.

Fusarium caudatum Wr. (one new variety)
F. culmorum (W. Smith) Sacc. (one new variety)
F. discolor Ap. et Wr. (one new variety)
F. falcatum Ap. et Wr. (one new variety)
F. Martii Ap. et Wr. (two new varieties)
F. metacroum Ap. et Wr. (one new variety)
F. oxysporum Schlecht. (three new varieties)
F. redolens Wr. (one new variety)
F. Solani (Mart. p. par.) Ap. et Wr. (two new varieties)
F. subulatum Ap. et Wr. (one new variety)
F. udum (Berk.) Wr. (one new variety)

The remaining organisms of the genus Fusarium were then named as new species and their varieties. It is probable that at least some of them have been previously described, but the descriptions given are so incomplete that any reasonable identification is doubtful. The matter of identification is discussed on another page.

Besides the Fusaria, several organisms were isolated in the course of the work which more or less closely resemble Fusarium though belonging to a different genus (Ramularia) of the Hyphomycetes. As these organisms are often found on the potato and are easily confused with species of Fusarium, they are treated here, but of course under their proper generic name.

On the whole the work, with a few exceptions, fully verifies the principles laid down by Appel and Wollenweber (1910). The chief exceptions are as follows: (1) The Fusaria as such can be distinguished on almost any medium, including artificial media, provided that the medium is not extremely poor or rich in food materials and also provided that the moisture supply in the medium is well regulated. (2) The microconidia should be recognized as a special kind of spores because of their importance in classification of these fungi. Though microconidia genetically do not represent a separate kind of spores, nevertheless their typical absence for certain closely related Fusaria, as well as their typical presence for others, is characteristic. Besides, whenever they are present they have a constant and often distinctive type of their own. (3) No typical coremial nor typical pionnotal form of fructification was observed in any of the true Fusaria, though types of fructification resembling those forms more or less closely were common.

SCOPE OF WORK

The work was confined to a study of the Fusaria as such, that is, without any consideration of the possible perfect forms.[3] The study was centered mainly on the characters that seem to be most important and practicable for a natural classification of these organisms. Thus, especial attention was paid to the presence and type of each kind of spores (micro- and macroconidia and chlamydospores). Type of color production was found to be next in importance. Type of fructification and of conidiophores, size of spores, presence or absence and kind of sclerotia, and rate and character of colony growth, were also found to be of more or less considerable importance for differentiation of the species.

Some work has been done on the action of these organisms under different environmental conditions and also with respect to their pathogenicity. In regard to the latter the following four facts should be mentioned:

1. Extensive inoculations of potato plants with all the Fusaria presented here yielded negative results and would indicate that they are not wilt producers.[4]

2. Several series of inoculations of potato tubers showed (a) that a considerable number of the Fusaria can cause more or less rapid decay of the tubers, and (b) that most of the Fusaria readily produce rot only after the tubers begin to sprout.

3. The commonest rot-producing organism, at least in eastern United States, is *F. coeruleum* (Lib.) Sacc.

4. The inoculations into tubers seem to indicate that some species widely different morphologically may act similarly pathologically, and that other species very closely related from the morphological standpoint differ very widely in their pathogenicity.

The other results of this phase of the work will be mentioned only in those instances in which different action under different environment is of importance in identification.

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[3] In this study no special attempt was made to discover the perfect stage of any of these organisms. At the same time it is to be noted that under the condition of the work when a considerable number of "natural" as well as "artificial" media were used, none of the Fusaria produced a perfect stage.

[4] The writer does not mean by this statement that the Fusaria actually never produce wilt of potatoes, because it is possible that the negative results were due to lost virulence of the cultures used or to some other important factor that escaped attention.

SOURCE AND METHODS OF ISOLATION

Most of the species of Fusarium and related organisms presented here were isolated from potato tubers affected with dry or soft rot. A number of isolations were made also from discolored fibro-vascular bundles of potato tubers and from stems of wilted potato plants. A single organism, *Fusarium metacroum* var. *minus*, was isolated from a spot on the surface of a half-dead potato stem.

In a few cases the isolations were made by means of poured plate dilutions, but in the majority of cases they were made from affected tissues of the host. The affected part of the host was first thoroughly wiped with a piece of cheesecloth moistened in 0.1 per cent solution of corrosive sublimate, and then the "skin" was peeled off just above the affected part, or the diseased part of the plant was broken open so that the spot from which the isolation was to be made was not touched even with sterile utensils.[5] Four or five small fragments of diseased tissue were cut out with a sterile scalpel and transferred with a sterile needle to cooled poured plates of a suitable medium. When a rotted tuber showed a noticeable difference, in color or otherwise, in different regions of the decayed part, a separate set of plantings was made from each region. The actual isolation of more than one Fusarium from a single tuber shows that this precaution was, at least in some cases, worth while.

Several days after plantings were made, if any fungi were present they usually had made considerable growth and often allowed a preliminary macro- and microscopic comparison of the isolated organism with any others of the same series of isolations or of former ones.

In case a fungus thus obtained was different in some way[6] from the others, two transfers into test tubes of a suitable medium were made for further study. In order to make a culture from the start as pure as possible, these transfers were made from the very margin of the colony. But if in a colony there were apparently two or more organisms growing together, this method would invariably lead to isolation of the rapidly growing one and loss of the slower-growing one. In order to save the latter also, the plates with original plantings were kept for a long time after the first transfers were made, and now and then dilutions were made from the old colonies. In the majority of cases this work was useless, but in two cases there were isolated very slow-growing fungi — *F. dimerum* var. *Solani* and *F. udum* var. *Solani* — which otherwise would have been missed.

---

[5] Small instruments and glassware may be sterilized conveniently by storing them in a jar of 80-per-cent alcohol. When ready to use the excess alcohol is burned off by passing the instruments through a flame. When so treated the instruments are sterile, perfectly dry, and not too hot for immediate use.

[6] Rate and character of growth, color, and type of spores were at first almost the only characters on which these organisms were judged.

The cultures obtained by transfers from original plantings seemed to be, and usually were, pure cultures from the start. Nevertheless it was evident that some method of obtaining cultures from single spores must be employed before a comparative study of the organism could be profitably begun.

In those few instances in which a culture did not produce any spores for a long time and which appeared to be a mixture of more than one fungus, an attempt was made to separate the organisms by the mere planting of a small bit of the fungous growth in the center of a newly poured plate. In only one case was the result satisfactory. This was when a bit of mixed growth of *F. arcuosporum* and *Ramularia Magnusiana*, on being planted in a plate, produced from the start on one side a pure growth of one fungus and on the other side a pure growth of the other fungus. In all the other cases a culture, if transferred into several different media, sooner or later always produced a sufficient number of spores; and in order to obtain a pure culture the poured-plate method of dilution was invariably employed.

Considerable economy of time and labor was effected by placing a number of separate drops of sterile water in a sterile plate. By transferring spores from drop to drop, a drop is soon secured in which the number of spores is such that a small loopful transferred to a drop in another plate will contain only thirty or forty spores. The melted and properly cooled medium is then poured into the plate, and distribution of spores is effected by giving the plate a rotary motion before the medium has hardened.

The first observation of the dilution plates was made about a day later. At this time most of the spores had germinated and could be observed from the bottom of the plate with comparative ease. When a single spore at some distance from the others was located, it was marked with a circle of india ink. In this manner several spores were marked and the plate was left for another day, after which the colonies were usually large enough to be observed with the unaided eye. Transfers could then be made easily and surely. These transfers have been kept as the stock cultures. The dilution plates from which stock cultures were obtained were kept for several weeks longer, in order to see whether all the colonies were alike.

In only three cases, after the first dilution, did there appear to be different colonies present in the same separation plates. In one of these cases this was the result of a mixed growth of two distinct fungi. It is of some interest to note here that the mixed culture appeared to be a fine specimen of a pink fungus. On dilution it gave rise to a brick red fungus, *F. metacroum*, and a white one, *F. diversisporum*. In the other two cases the fungi obtained from the differently appearing colonies represented most closely related organisms which never were isolated again, but which nevertheless remain distinct from each other. Whether they represent mutations, fluctuations, or mere chance coexistence of the two actually different organisms, is a matter yet undecided. They are tentatively designated as two different varieties — *F. bullatum* var. *roseum* and *F. arthrosporioides* var. *asporotrichius*.

The stock cultures were subsequently repeatedly rediluted, and, as was to be expected, almost invariably[7] every dilution plate showed all the colonies from the same stock culture to be alike.

As it is not possible to use a high-power objective with the ordinary petri dish, and as single spores of some of the species are very difficult to locate because of their size and lack of color, there was always the possibility that some of the cultures which seemed to be pure were really mixtures, for it is a well-known fact that the spores of many fungi often cling together tenaciously. In order to make absolutely sure that pure cultures were being dealt with, the further precaution was observed in 1913 of locating single isolated spores with a high-power objective and securing cultures from them.[8] The cultures thus obtained were in all respects similar to the original stock cultures, thus confirming the reliability of the poured-plate dilution.

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[7] Only two exceptions were observed: (1) In the case of *F. metacroum* var. *minus* among characteristic red colonies, there appeared one colorless colony. For a while the absence of color was a constant character, but later the color was produced again. (2) In the case of *F. diversisporum* among colonies with high aërial mycelium there was a colony almost without aërial mycelium. In subsequent generations the aërial mycelium reappeared almost to the same extent as originally.

CULTURE MEDIA

It is a well-known fact among mycologists that under different environmental conditions many fungi vary considerably in their macro- and microscopic characters. One of the most important factors in variability is the substratum. According to Thom (1910), certain characters in Penicillia appear only when the fungi are grown on a certain medium. Other instances of a similar nature might be cited.

Because of this variability of many fungi with variation in the substratum, it seemed indispensable in this study of Fusaria to use a number of different media in order to find the extent of the variability in the Fusaria and to determine, if possible, which media could be most profitably employed in this kind of work.

The so-called " natural " media, as well as artificial media, were used.[9] Those employed most extensively were potato tuber and stem plugs, and hard agars of potato, lima bean, and oat. For the study of color production, from 8 to 10 per cent of sugar (glucose) was added to one of the above agars, usually to potato agar. In all other cases the agars were used without glucose or with a small amount of it (0.5 per cent). In a few instances the agars were more or less acidified by the addition of small quantities of lactic or citric acid (from 1 to 3 drops of 50-per-cent acid to 10 cubic centimeters of the medium); otherwise they were used as they were after the process of cooking and sterilizing.[10]

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[8] The method employed was as follows: A number of drops (from eight to ten) of sterilized potato broth were placed on a sterile glass slide. Dilution transfers were made from drop to drop until such a dilution was secured that on removing a small droplet on the flattened end of a platinum needle it was found by microscopic examination that in many cases a single spore could be obtained. From such a drop nine transfers were made to a sterile, but somewhat greasy, cover glass. By placing the cover glass over a tubular glass cell or a van Tieghem cell, each individual droplet could be examined with a high-power objective. Droplets containing no spore or more than one spore were wiped off at once with a pointed piece of blotting paper. In thus removing spore droplets, the spores also were invariably removed. Sterile water was placed in the bottom of the cell and the cover glass was sealed to the cell with sterile water. The cells were then placed in a moist chamber for about twelve hours, and by this time the spores had usually germinated and could be observed with much greater ease. This observation was almost indispensable in the case of those species that produce numerous minute microconidia, as any such could be easily detected at this stage. Only two or three droplets bearing single unmistakably germinating spores were allowed to remain on the covers. At the end of twenty-four hours more, the growth of mycelium from the single spores was usually sufficient to be seen with the unaided eye and could be transferred readily with a finely pointed needle to a suitable medium.

[9] The media used were: (1) Natural — potato, bean, and pea stems; rye straw; canes of red raspberry; grains of rye, wheat, oat, barley, corn, and rice; corn meal and oatmeal; whole potato tubers and plugs of potato tuber. (2) Artificial — potato, lima-bean, oat, corn, and nutrient agars (hard and soft, from 1 to 3 per cent agar, neutral and more or less acidified, without and with different amounts of glucose).

The method of preparing all natural media was very simple. It consisted in cutting suitable pieces, tubing, and sterilizing in an autoclave for twenty minutes at fifteen pounds pressure. Enough distilled water was added to the tubes to keep the cultures in good growing condition for from three to four weeks, the exact amount, of course, depending on the size and succulence of the material used. The decoctions that were most commonly used were prepared in the following ways:

Potato decoction.— Two hundred grams of peeled potato tubers were sliced, 1000 cubic centimeters of distilled water was added, and the material was cooked for about forty minutes in a double boiler. The clear liquid was then decanted and the volume restored.

Lima-bean and oat decoctions were prepared by soaking 100 grams of ground lima beans or oats in 1000 cubic centimeters of distilled water at 60° C., in an incubator for an hour. The liquid was separated by straining through cheesecloth and the volume was restored to the original 1000 cubic centimeters.

Corn meal decoction was prepared as described by Shear and Wood (1913); that is, from about 35 to 40 grams of corn meal were treated in the same way as 100 grams of lima beans or oats in the preceding case.

For the artificial media, to 1000 cubic centimeters of a decoction were added agar (10, 15, or 30 grams, these amounts corresponding respectively to soft, medium, and hard agar) and glucose (0 to 100 grams), and the whole was cooked in a double boiler over a free flame for an hour, or, more exactly, until all the agar was well dissolved. The liquid was then tubed, plugged, and sterilized in the same way as were the natural media.

At the present stage of this work, it seems that all the labor spent on using so many different media was more or less wasted. It seems that the same results could as well be obtained with but a few good media, preferably the following: a hard oat agar (without glucose), a stem and tuber plug, and a potato agar with about 5 per cent of glucose. All other media used in the course of this work did not prove to be of any specific value, and at the best gave the same results that were obtained with those just named.

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[10] When an acid was added to an agar this was done after the medium was sterilized, the medium then being quickly cooled. This quick cooling was necessary because otherwise an acidified agar often would not solidify as well as it should.

For determination of the color of the substratum and also for study of the colony growth, it would perhaps be best to use poured plates with about 10 cubic centimeters of a clear agar. In this work potato hard agar with from 5 to 10 per cent of glucose was almost exclusively used for this purpose. For the study of other characters, in most cases cultures were made in common test tubes.

EFFECT OF VARIOUS MEDIA ON DIFFERENT CHARACTERS OF FUSARIA

In the course of this work it was observed, in partial confirmation of the statement by Appel and Wollenweber (1910: 12–23), that certain media affect fungous growth more or less characteristically. A medium too rich in nutrients, especially in glucose, usually gives cultures with more or less abnormal spores, the abnormality showing itself in a too dense granulation of the protoplasm, in more or less considerable swelling of the spore cells, and often in abnormal septation, size, and shape.

Media rich in glucose usually increase the density of color produced by these fungi, and often also change its character. For example, a pink fungus, *F. arcuosporum*, is turned to a clay-colored one; or the fungus *F. angustum*, which is colorless or nearly so, is turned to a more or less bright purplish-vinaceous one; and so forth.

Excess of water in a medium usually leads to comparatively quick degeneration (self-digestion?) of the spores, and, in general, to a shortened duration of the vitality of the culture. Its presence, at least in case of a soft agar as compared with a hard agar, is usually unfavorable for the normal development of aërial mycelium.

A medium comparatively poor in nutrients, such as corn meal agar, seldom gives rise to sclerotia and plectenchymic sporodochia; but in a way it is a good medium for the study of chlamydospores, which are produced here more or less freely and stand out more clearly than in other media.

Whole potato tubers (steamed) often are most favorable for production of large sporodochia; this medium, and also potato tuber plugs (also steamed), show the largest sclerotia.[11]

An agar, especially such a one as oat hard agar, often gives all the forms of fructification for these fungi, with " normal " spores and more or less typical and brilliant color production.[12]

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[11] See also Wollenweber (1913 a: 25).

On the plugs made from stems of different plants, spore production seems to be normal.[13] The spores are of typical and comparatively uniform shape, septation, and size, without too dense granulation of the protoplasm, and with a long duration of vitality. It often is found, however, that on such media spores are less normal than, for instance, on a hard agar.

Presence of the epidermis on the stem and tuber plugs seems to favor production of fewer but better developed sporodochia,[14] and often lessens development of aërial mycelium.

Certain media, such as boiled rice, give a color that is typical for certain related Fusaria.

EFFECT OF LIGHT AND OF REACTION OF THE MEDIUM ON DIFFERENT CHARACTERS OF FUSARIA

As was stated in the introduction, very little systematic work has been done on the ecology of the Fusaria. The few things which have been found by other workers and by the writer, and which have some value in the determination of these fungi, may be summed up briefly as follows:

1. A diffuse daylight may affect color production considerably (Smith and Swingle, 1904:48–49), but in most cases the effect is only slight or there is almost none. It usually intensifies the colors produced by these fungi. A very strong light often makes the colors somewhat duller, especially in the case of Fusaria producing bright red colors, these being turned toward brown hues. No noticeable effect of the light was observed on other characters of these fungi, although Appel and Wollenweber are of the opinion that the spores are of a more normal type in the light than in the dark.

2. According to Appel and Wollenweber the reaction of the medium has an especially noticeable effect on blue colors, which can appear only in a medium of neutral or rather alkaline reaction. The blue color in a medium of an acid reaction will appear as orange. True red colors remain

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[12] This observation is apparently in some contradiction to the observations of Appel and Wollenweber (1910:12–13), but indeed it is not so; because, judging by the " artificial " media actually used by them, their observation of unfitness of such media for study of " normal " growth of the Fusaria was based on " soft " agars too rich in sugar. The writer also found that such agars produced abnormal growth.

[13] This was observed first by Appel and Wollenweber (1910), and on this observation mainly they concluded that the only way to study the Fusaria properly is to study them on such " natural " media.

[14] This was first observed by Appel and Wollenweber (1910).

red even in a medium of an alkaline reaction. The observations of the writer, so far as they go, are in full agreement with the above statements; to which it may be added that certain fungi which in a neutral medium produce a grayish white aërial mycelium (*F. sclerotioides*), in the same medium strongly acidified (0.4 per cent by weight of citric acid) produce an aërial mycelium of a pink-vinaceous shade.[15]

It was observed also that acidity of the medium lowers the rate of the fungous growth and makes zonation of the colony more prominent and closer. The retardation of the growth depends on the kind of acid (see also Smith and Swingle, 1904:42, 48) and its concentration; different Fusaria are not affected in the same degree, some tolerating more acid than others (see also Lewis, 1913:238).

THE GENUS FUSARIUM

Appel and Wollenweber (1910:4–12, 23–61) present a very detailed, critical study of the genus, with the following list of synonyms:

Atractium Link pr. p. (1809)
Fusidium Link pr. p. (1816 and 1825)
Fusisporium Link (1824)
Selenosporium Corda (1837)
Fusoma Corda (1837)
Pionnotes Fries (1849)

The description of the genus Fusarium given by these investigators, when other data presented by them are also taken into consideration and using the terminology of Lindau (1905, 1908–1909), may be stated in brief as follows:

Hyphomycetes of Mucedinaceæ-Hyalophragmiæ, Hyalostilbaceæ-Phragmosporæ, and Tuberculariaceæ-Mucedineæ-Phragmosporæ groups, with smooth, not appendiculate, mostly sickle-shaped, acrogenous, noncatenulate conidia.

The genus as delimited by these authors was to include also forms such as *F. dydimum* and *F. Willkommi*, with cylindrical, one-septate spores, and bacilliform, one- to five-septate spores.

The writer has not sufficient material on hand to warrant any change in the above characterization of the genus; but in the course of this work, a true Fusarium never was observed which would produce a typical coremium[16] or a typical pionnotes,[17] that is, the two fruiting forms on the basis of which the genera Atractium and Pionnotes were founded by their authors and which are now reduced to synonymy by Appel and Wollenweber because these investigators find in the Fusaria certain structures resembling these two forms.

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[15] The medium used in the instance cited was potato hard agar plus 5 per cent of glucose. The cultures were made in petri dishes.

That one of these forms, Pionnotes, is a distinct form, and that its characters can be used as a basis in a classification of Fusarium-like organisms, has been shown lately by Wollenweber. After citing *Fusarium* (*Pionnotes*) *udum* and its variety as instances of the Fusaria without "Fusszelle," he (1913 c: 206) says: "Beiläufig bemerkt, bilden solche Fusarien zusammen mit *F. aquaeductum* eine gute Section der Gattung, die ich Eupionnotes nenne wegen des Übergewrichtes dieser Sporenverlagerung."

There is very little doubt that a true coremial form of fructification is quite distinct, and does not occur in the species of Fusarium observed by the writer.

With respect to the present status of the genus Fusarium, some recent changes must be considered here. Wollenweber (1913 a:33) transferred from the genus Fusarium to the genus Ramularia all forms with conidia of *F. dydimum* type when chlamydospores are present.[18] The same author (1913 c:225), somewhat later, also excluded from the genus Fusarium forms with conidia of the type of *F. Willkommi* and transferred them into a new genus, Cylindrocarpon,[19] established for this purpose. Thus, in fact, all forms having cylindrical conidia or conidia with rounded ends are excluded by Wollenweber (1913 c:239) from the genus Fusarium. The genus Sepedonium Link (1809), according to Wollenweber (1913 c: 200), is only a chlamydosporial stage of *Fusarium orthoceras*.

It appears, then, from the study of the Fusarium-like organisms of potatoes (which, on the whole, represent a great diversity of forms) and also from a careful survey of the genus Fusarium as it stands in literature, that the following characters are of generic value:

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[16] The terminology which is used here is the same as that of Lindau.

[17] Only one Fusarium-like organism was isolated from potatoes which produces a fruiting layer very similar to a pionnotes (*F. udum* var. *Solani*), but it is very distinct from all the other Fusaria and can be used rather to support than to disprove the above statement.

[18] In those cases in which chlamydospores are not present, Wollenweber says the fungi have a perithecial stage of the genus Mycosphærella.

[19] This genus is to include only those forms for which a perithecial stage has not yet been found. *F. Willkommi* has been connected with *Nectria galligena*.

1. Color of conidia and mycelium, never of a plain gray or black color but mostly of various brilliant hues.

2. Conidia dorsiventral,[20] attenuate, pedicellate,[21] not appendiculate, smooth, normally not constricted at the septa, distinctly three- (or more) septate,[22] acrogenous, not in chains.[23]

3. Conidiophores with single to irregularly whorled branches, never truly dichotomous nor of a strictly penicillate or verticillate type. The conidiophores, through many times repeated branching or also by growing side by side with other conidiophores, typically give rise to macroscopically observable, dense tufts of conidiophores covered more or less deeply with a somewhat slimy mass of spores. Such fruiting bodies, tuberculate in form (sporodochia),[24] may be with or without a plectenchymic (=pseudoparenchymic), flat or wartlike base, without any differentiated enclosure.

4. Chlamydospores (endogenous, double-walled, resting bodies) terminal and intercalary, or only intercalary,[25] or none, and produced both by mycelium and by spores.

5. Mycelium composed of hyphæ which are always distinctly, but not closely, septate, and irregularly, never dichotomously, branched, the secondary branches usually thinner than the primary ones; protoplasmic content of the mycelium for the greater part plainly present and usually distinctly vacuolate. The rate of growth in artificial media, when compared with the Fusarium-like organisms studied, is comparatively high.

It must be added here, as a general remark to the characteristics of the genus Fusarium given above, that in this case, as well as in any other attempts at classification of natural phenomena, the boundaries laid down for separation of one group of phenomena from all the rest have only a relative value. Thus an organism may deviate[26] in a greater or less degree in one or a few of the characters given above, and yet, so to speak, be a good Fusarium provided all other characters of this genus are well expressed by the organism.

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[20] This term is used by Appel and Wollenweber (1910).

[21] This term is used by Wollenweber (1913 c). See his key on page 219 of reference cited.

[22] Fusaria-like organisms with one-septate conidia are rare. Two such organisms were isolated from potatoes, and from over one hundred and sixty Fusaria recorded by Lindau (1908–1909 : 517–588) only seven are definitely stated to have one-septate conidia; and of these seven at least two are undoubtedly not Fusaria if the changes made in this genus by Wollenweber are considered.

[23] Microconidia may be produced in chains. This is true in case of certain Fusaria of corn and of coniferous seedlings. (See Sheldon, J. L. A corn mold [Fusarium moniliforme n. sp.]. Nebraska Agr. Exp. Sta. Rept. **17**: 23–32, fig. 1. 1904.)

[24] The terminology used here is the same as that used by Wollenweber (1913 a : 24, footnote).

[25] The only Fusarium-like fungus isolated by the writer which has only terminal chlamydospores seems not to be a typical Fusarium. (See *F. cuneiforme*, key and description.)

[26] Of course a true Fusarium, evidently, in no case can be of a plain gray or black color (in mycelium and conidia as well), or have non-septate or cylindrical macroconidia with both ends rounded. (In regard to the macro- and microconidia, see page 116.) Its conidia cannot be appendiculate nor its conidiophores of a true verticillate or any other specifically peculiar type.

VARIABILITY IN THE FUSARIA

Many of the morphological and physiological characters of the Fusaria show marked variability, and at first seem to be of such a nature as to discourage any attempt to treat these fungi on a morphological basis. Thus Smith and Swingle (1904:27), in regard to their *F. oxysporum*, say: "......This fungus showed a number of very striking variations. For this reason it is impossible to give a general description that will hold universally." And after quoting descriptions of eleven old species of Fusarium from potatoes, the authors conclude as follows (1904:51): "Judged by the above descriptions, we have had a half dozen or more species of *Fusarium* in our culture tubes, some of them 'new species,' and yet all were the product of a single spore. This does not mean that there have been in our cultures any very wonderful transmutations of one thing into another, but only that organisms respond to their environment, and that 'species descriptions' of the kind cited have not taken this fact into consideration, and consequently are worthless for scientific purposes. This is not a new idea, but it is a fact to which the attention of systematic mycologists might be directed profitably at frequent intervals."

Sometimes variability, for example in the type of conidia, is so great that a student gives up hope of determining the actual type. Thus Wilcox, Link, and Pool (1913:24) conclude their discussion on the form of the conidia produced by their fungus[27] as follows: "All sorts of stages are shown in the plate, so that each one can judge for himself, as it is possible that one who has studied a great number of species of *Fusarium* will be able to pick out the characteristic form which can be set aside for this particular species."

A great number of instances of variability in this group also could be cited easily from the species presented in this paper. But it will suffice to state here only those instances of variability which are, perhaps, most important and more or less common:

1. The type of the conidium varies in many Fusaria from micro- to

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[27] *F. tuberivorum* Wilcox and Link, which according to Wollenweber (1913 c : 206) is identical with *F. trichothecioides* Wr.

macroconidia, and both sometimes are of a very diverse type even within themselves. (See *F. diversisporum.*)

2. Conidiophores often vary from mere minute projections on the side of a hyphal thread to a complex dendroid structure often of a size observable macroscopically.

3. In the Fusaria for which production of sporodochia, plectenchymic stromata, or sclerotia is typical, it sometimes happens that they are not produced even under apparently favorable conditions. These structures may vary in size, number, and form.

4. The presence or absence of color is an especially variable character. Changes in type of color may occur, but such changes are only apparent and in all known cases can be explained on the basis of the difference in the reaction of the medium (certain orange-red colors in acid become blue in an alkaline medium), or, when a typical color is a compound one, the change may be due to the preponderance of one or another of its elementary colors.

5. Extent of the development of aërial mycelium, zonation, rate of growth, and character of the margin of the colony, may also vary to a considerable extent.

6. Especially great variation may take place in the relative production of the different types of conidia and chlamydospores.

It might appear at first glance that the variation of these fungi is so great as to leave no firm ground for morphological treatment of the group. And yet the actual situation is far from being so hopeless. First of all, the great majority of the variations mentioned above occur, as did those observed by Smith and Swingle (1904:59), under different environmental conditions; while the variability under identical conditions usually (see also Lewis 1913:225) is very slight. Secondly, the cases of variability cited above are primarily cases of variation between presence and absence of certain characters — which is, after all, of no great importance, because when a certain character is present it is always peculiar to a specific type of organism and thus this organism can be separated from others. Then there are certain Fusaria which under almost any but extreme environmental condition remain nearly the same throughout (as *F. udum* var. *Solani* and *F. cuneiforme*). And finally, the most important character in the classification of these fungi — the type and the shape of the conidia

— is after all sufficiently stable to be used safely in a morphological treatment. Even the size of conidia, when a sufficient number of measurements is made and averaged and when only conidia of the same type are compared, is of rather surprising uniformity and stability. It is believed that every one will agree with these statements after an examination of the data presented here in the descriptions of species.

That Fusaria can be separated on a strictly morphological basis was first definitely demonstrated by Appel and Wollenweber.

RELATIVE TAXONOMIC IMPORTANCE OF DIFFERENT CHARACTERS

Usually the particular organism and the prominence and stability of a given character determines the relative importance of characters in classification. Thus, in certain cases septation of conidia may be considered as the important character. For instance, in the case of *F. dimerum* and *F. affine*, this character distinguishes these two fungi at once from all the other Fusaria, while in most other cases it is not of such importance. Presence of a continuous slimy layer of spores is the important character in the case of *F. udum* var. *Solani* where this type of fructification is especially prominent. Type of microconidia, when they are of typical occurrence and especially when they are of a peculiar type, as in the case of *F. sporotrichioides*, is also a most important character. Type of chlamydospores is an important character in general for dividing the whole genus into sections, but it usually has no specific value; and yet there is a fungus, *F. cuneiforme*, which can be set off from all the rest because of its terminal unicellular chlamydospores. Color may vary considerably, but on the whole its type is stable enough to be of considerable help in dividing Fusaria into sections, and sharp contrasts in color can often be used for specific differentiation.

In general the most useful and evidently sure basis for a natural classification of this group is the shape of the macroconidia.[28] Their dorsiventrality, the form of their apex and their basal cell, and their septation, and also their size, when properly used, are of considerable service in separation of species. The actual working value of each character can be seen from the keys.

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[28] Appel and Wollenweber (1910 : 34) and Wollenweber (1913 a : 26) came to this conclusion long before the writer. See also Lewis (1913 : 225).

FORMS OF FRUCTIFICATION IN PURE CULTURES

Spores

The spores in the genus Fusarium are of two kinds: conidia, or acrogenous spores, and chlamydospores, or endogenous resting bodies. The conidia in turn may be divided into two more or less distinct types: (1) macroconidia, sickle-shaped, three- or more septate spores; and (2) microconidia, oval, non- or only one- or two-septate spores.

Before the appearance of Appel and Wollenweber's work (1910) the existence of micro- and macroconidia as two distinct types was more or less generally accepted, but in that work it was concluded (page 29) that there is only one type of conidia. The latter view is, of course, correct so far as the genetic relationship between micro- and macroconidia goes. Nevertheless, the typical presence of microconidia in certain closely related Fusaria and their typical absence in others is characteristic. Besides, whenever they are present they have a comparatively constant and often peculiar type of their own. Thus, they can be used as a good natural basis for classification of these fungi, and for that reason must be considered as a type separate from macroconidia.

In this paper the term *microconidia* is applied to all nonseptate, and seldom to one- and even to two- or three-septate, conidia of a different shape from that of the macroconidia, which are sickle-shaped and usually three- or more septate. Different forms of macroconidia are shown in figure 1 (page 112).

Chlamydospores may be borne on the ends of special lateral branches of the mycelium (terminal chlamydospores), or they may be intercalary. They often are produced in the ends of the conidiophores, in the conidia or in the ends of special branches from the conidia. The chlamydospores are single, in short to long chains or in more or less large clusters. They are of common, though not of general, occurrence, and in a number of Fusaria both terminal and intercalary chlamydospores are present; in some others there are only terminal, and in still others only intercalary chlamydospores have been observed. A number of Fusaria evidently have no true chlamydospores; they may possess structures with dense content, but these structures are not thick, double-walled bodies.

Forms of fructification

Sometimes, as in the case of the conidia on aërial mycelium of *F. trichothecioides*, and also in the sporotrichial form of *F. sporotrichioides*, the conidia are borne singly and remain thus in the form of a powder. Usually, however, they adhere to the tips of conidiophores for a time, forming balls of variable size. This may be the case with microconidia as well as with macroconidia. In certain Fusaria, such as *F. cuneiforme*, and in all members of the section Martiella, these balls of conidia are especially prominent; but they are very common also in the other Fusaria and therefore cannot be used for specific differentiation.

When there is no room for old conidia to be pushed aside — that is, when the fruiting branches are very numerous and close together — a considerable and continuous mass of spores results; these, when the air is moist, form a roundish, wartlike heap of spores, which, with the conidiophores producing them, is known as a **sporodochium.** When the air is comparatively dry, the spores are pushed up in more or less curled, long, tendril-like columns. This is often observed especially in many cultures on sterilized canes of red raspberry and in stem plugs of other plants.

Often small sporodochia are produced on and strewn all over the aërial mycelium, producing a picture very characteristic for *F. subulatum* and some other Fusaria. In other cases minute and numerous sporodochia are produced very close to or on the surface of the substratum, and when these minute sporodochia are very numerous they form a nearly continuous, slimy layer of conidia. The fructification then resembles a pionnotes and is called here **pseudopionnotes.** In one case a seemingly true pionnotes[29] — that is, a thick, continuous, slimy layer of spores — was observed (in *F. udum* var. *Solani*). A pseudopionnotes may be produced under aërial mycelium which may more or less mask its macroscopic appearance, as in the case of several Fusaria of the section Elegans; but in certain cases there is no aërial mycelium over it, and the pseudopionnotes remains fully exposed and characteristic for certain species. (See *F. metacroum*, *F. falcatum*, and *F. discolor* var. *sulphureum*.)

Mycelial threads in many Fusaria often run parallel and anastomose more or less closely, thus producing a ropelike structure which may come up into the air in an irregular fashion and which also may bear more or less abundant conidia produced on side branches. These ropelike structures then resemble coremia, which are columnar fruiting bodies, typical of the family Stilbaceæ. No true coremia were observed in any of the Fusaria presented here.

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[29] The definition is taken from Lindau (1908–1909: 509).

A stroma, in the sense employed here,[30] is the fungous layer on which aërial structures (aërial mycelium, conidiophores, and spores) are produced. The stroma may consist of more or less loose hyphæ, or it may form a dense pseudoparenchymic (plectenchymic), continuous sheet, fleck, or prominent wart. There are often produced also roundish, more or less wrinkled, often shiny, bodies, resembling true sclerotia. They are of a dark blue color, and among the Fusaria studied in the course of this work they were observed only in certain species of the section Elegans; though according to Wollenweber (1913 a:32) they are very common also in the section Roseum, and are characteristic for one species, *F. sclerotium* Wr., of the section Gibbosum.

METHOD OF STUDY AND PRESENTATION

The method actually employed in this work consisted in the cultivation of pedigreed strains of the various organisms on various natural and artificial media. Almost without exception, all the strains were transferred to a new medium on the same day and the whole set of cultures was kept under the same environmental conditions. In all important cases duplicate cultures were employed. For the inoculum, as far as possible, similar material was used — that is, only mature spores or only aërial or only submerged mycelium. The importance of the same environmental conditions for a comparative study is evident. It applies equally as well to the kind of inocula used, as it was found[31] that often an inoculation made with spores tends to better spore production, and an inoculation with mycelium often results mainly, at any rate at first, in the production of mycelium.

In order to bring all cultures to the same stage of maturity and also to assure their purity, dilutions in poured plates were made again and again for the entire set of the Fusaria (of isolations made by the writer, as well as of the organisms obtained in culture from other sources), and then new transfers were made from colonies about two days old produced in the plates.

In the macroscopical examination of the culture, special attention was given to the presence or the absence, and the character, of aërial mycelium; to the kind of fructification layer (pionnotes-like, sporodochial, and so forth); to the color of spores, of aërial and submerged mycelium, and of substratum; and to the production of special structures such as plectenchymic stromata and sclerotia.

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[30] A slightly different definition of stroma is given by Wollenweber (1913 a: 24, footnote).

[31] See Appel and Wollenweber (1910: 13), and also Lewis (1913: 209). The same was frequently found to be the case also in the course of this work.

In the microscopical study, the different types of conidia, chlamydospores, and conidiophores received special attention. The observations were recorded by means of camera lucida drawings[32] and necessary measurements.

When measurements were of any importance,[33] ten conidia (or chlamydospores) were measured and only the average and the extremes of these measurements were recorded. In cases of special variability of the material, records were made of fifteen or twenty spores of each important type of septation and shape.

In making camera lucida drawings, care was taken to picture the apical and the basal ends of the conidia with the utmost accuracy; every kind of conidium occurring in a culture was drawn, but the typical and the exceptional cases, as they appeared, were marked off.

As a rule, measurements and drawings usually were made of the conidia taken from sporodochial or pionnotes-like masses of them, because such conidia on the whole are more uniform and typical for a given organism. Here it should be noted that for the measurements and drawings it is highly important, at least in case of very closely related organisms, to use material analogous in all respects — age, type of fructification, and environmental condition.

The fungi were studied, not only on different media, but also at different stages of their growth. The latter factor is almost as important as the former, because in certain cases, as in *F. angustum*, conidia in the best condition (most regular, and so forth) were observed when the cultures were very young. Some characters, however, become manifest only after a culture has reached a certain stage of maturity. This is often the case with chlamydospores and color production. Sclerotia often appear in comparatively old cultures and continue to grow for some time.

The presentation of the species of Fusarium is based on the following main principles:

All drawings (with only a few exceptions) are intentionally made to the same scale as those of Appel and Wollenweber — thus far the one fundamental work on this subject[34] — so that comparison can be made easily. The drawings represent all types of conidia, abnormal ones included, so that no one may be misled by types actually observed by him and by those given here. This might easily be the case if only normal material were presented, because our understanding of what is normal is very relative, is often too broad, and seldom corresponds to the things as they are. The particular forms that seemed to be normal are indicated by the drawings in figure 1 (page 122).

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[32] All drawings were made from living material mounted in water, and, with a few exceptions, with an oil immersion lens. In order to prevent movement of the spores in water, it is necessary first to use just enough water to keep air from underneath the cover glass, and then to spread around the cover glass some oily substance, as cedar oil, which does not dissolve in water and which does not dry out quickly.

[33] A number of fields should be examined before measuring in order to see the prevailing type, and then measurements of the conidia of the prevailing type should be taken. This leaves much to personal interpretation, but otherwise it would be necessary to take many more measurements.

Usually no attempt was made to represent the structure of the cell content, because it was not considered of taxonomic value. In those few cases in which the structure of the plasma seemed to be characteristic it is shown in a very approximate way.

The microscopical character of the mycelium, so far as observed by the writer, cannot be used for specific differentiation of these fungi; therefore no attention has been given it, either in drawings or in measurements, except in a very few instances when certain striking peculiarities have been so recorded (Figs. 34M and 43L).

In giving measurements of the size of spores, it was considered necessary, first, to give an average size for conidia from a particular culture, as well as the average of the measurements for the entire series of cultures. Thus the measurements have a definite meaning and are comparable with one another, provided the measurements are taken separately for each type of spore.

In arranging the species according to their relationship it was found convenient to follow Wollenweber's example (1913 a:26–27), and divide all the Fusaria into sections. Most of the sections are the same as those of Wollenweber, but because of some organisms presented here that could not be placed in any of his sections, certain new sections have been established provisionally.

In connection with taxonomic study and presentation of the Fusaria, it is necessary to discuss, at least briefly, the conception of the so-called normal culture. The observations of the writer in this regard are principally of the same nature as those of Appel and Wollenweber (1910:21–22) and can be summarized as follows:

1. A culture can be in the state of undevelopment (*Ankultur*) when the growth shows mycelium to the entire exclusion of, or to an abnormally

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[34] It is firmly believed that standardization of the subject presented is especially important in the study of this difficult group of fungi.

poor production of, spores; other forms normally present in the culture may also be absent. This state of culture may sometimes exist when a fungus is transferred from a mycelial growth, especially when it is taken directly from host tissues.

2. A culture is considered to be a normal one (*Normkultur*) when all forms typical to the fungus — and especially the most important form, the macroconidia — are abundant, comparatively uniform in size and shape, smooth in outline, and so forth.

3. After a long cultivation on artificial media, a fungus may lose certain characters, such as ability to produce certain color and also its virulence as a parasite. Such a state of culture is that of degeneration (*Abkultur*), and it may be accompanied also by smaller size and abnormal septation of the conidia. (Only loss of color was observed by the writer; the other observations are those of Appel and Wollenweber, 1910:22.)

4. In the first period of growth of any one of the Fusaria, the first conidia produced usually soon begin to produce new conidia, sometimes on minute papillæ located directly on the conidial walls, and sometimes on more or less well-differentiated and well-developed conidiophores, the process often much resembling the budding process in yeasts. In such young cultures the conidia are more or less swollen, their contents are commonly densely granular, and septation is not clear. This period (*Jungkultur*) may last from one to five or more days.

5. After the period just described, the fungus, when in normal condition, produces conidia which remain for a greater or a less length of time, of perfectly smooth outline and with clear septation. At this state of maturity (*Hochkultur*) the conidia are also of most uniform and typical shape and size, and it is the important stage for taxonomic study of these organisms.

6. Finally there comes a period (*Altkultur*) in which the conidia begin to disintegrate through the process of self-digestion, or at least become of less uniform and perfect type, and after a period of time the culture begins to lose its vitality.

These variations in character of the cultures show that only normal and mature cultures must be considered, though the character of other conditions may also give some help in identification. Another point which must be clear from the account given above is that in the study of these fungi they must be grown for a long period of time, under different cultural conditions and from different kinds of material for inocula. Then, after observation of all the stages, it will be possible to pick out easily the typical and normal one and base judgment mainly on that. Most minute attention must be paid to the shape of macroconidia.

In order to avoid much of the possible confusion in regard to the color production by the fungi, it will perhaps be not out of place to mention again that a convenient standard of colors must be used. Ridgway's (1912) color standards have been used in this work.

IDENTIFICATION OF THE FUSARIA WITH PREVIOUSLY DESCRIBED SPECIES

An identification of the Fusaria of potatoes with Fusaria formerly described as occurring on other substrata and even on potatoes, is in most cases impossible or at least rather doubtful. The species have been described mostly from material as it was collected in nature. After what has been said in the foregoing pages about variability of the Fusaria, about their common occurrence in different, often abnormal, stages, about the necessity of most minute attention to the peculiarity of the shape of the macroconidia, and about the importance of good drawings and of measurements properly made and presented, it can easily be seen what small chance there is of identifying an organism from any typical description of one of these species. The figures are often absent, and when present they are either too schematic or so inaccurate that they might just as well be omitted. Any citation in support of this statement would be superfluous.[35]

There are a number of different Fusaria which agree with a certain description and therefore might be considered as the same species. At the same time the very same organism may have other characters which, if studied alone, would surely set it off as a distinct species. An extensive illustration of this state of affairs is given by Appel and Wollenweber (1910:9–12), but the most impressive case is that of Smith and Swingle (1904:50–51). Therefore, although the literature on the subject was fully examined, especially publications of descriptions supplemented by any kind of illustrations, usually no definite conclusion in regard to identity could be arrived at. Because of this, and also because the most important literature is already listed in a few works on this subject (especially by

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[35] Appel and Wollenweber (1910: 12) in this connection made the following statement: "Oft blieb uns daher weiter nichts übrig, als neue Namen zu geben und ihnen so genaue Beschreibungen beizufügen, dass nunmehr die Arten immer wieder erkannt werden können."

Appel and Wollenweber in 1910, and by Wollenweber in 1913), this literature is usually not cited and a bibliography is omitted.

The condition of the taxonomic literature on species of the genus Fusarium in general is characterized by Wollenweber (1913 a:41) as presenting "an almost hopeless confusion." The only favorable exceptions known to the writer are the works of Wollenweber, alone and in association with other authors. These works are fully considered here. In fact there is hardly any difficulty in recognizing Fusaria described by Wollenweber. Nevertheless all available cultures of his organisms were grown along with the other Fusaria, and a thorough comparison of living material was made whenever it was necessary.

When this work was actually completed there appeared the work of Lewis (1913) on certain disease-producing species of Fusarium. The work was supplemented also with Wollenweber's list of the names for the fungi studied by Lewis, and also with certain remarks by Wollenweber in regard to the taxonomy of those organisms. It appeared that certain of Lewis's organisms were the same as those isolated by the writer from potatoes. Cultures of four Fusaria, somewhat resembling certain species isolated from potatoes, were obtained by the writer through the courtesy of Dr. Morse, Plant Pathologist in the Maine Agricultural Experiment Station. On comparison these four organisms — *F. pirinum* (Fries) Sacc., *F. conglutinans* Wr., *F. citrinum* n. sp., and *F. argillaceum* (Fries) Sacc.— were found to be distinct from all Fusaria presented here.

SYSTEMATIC ARRANGEMENT OF THE SPECIES OF FUSARIUM OF POTATOES

The natural key to the species and their arrangement in sections,[36] as given here, are in the main similar to those of Appel and Wollenweber (1910:59–60) and of Wollenweber (1913 a:28–32), respectively. Some changes and additions have been made, however, in order to include many new organisms.

Variety is used as the smallest unit in the taxonomic treatment of these fungi. An organism is classed as a variety if it differs from the closely related species in only one character, even though the difference be conspicuous; the same treatment holds with respect to the organisms that differ slightly in a few characters. When the differences are slight, but in several important characters, the organisms are classed as distinct species. In general it was preferred rather to name a possibly good species as a variety than to raise a mere variety to specific rank.

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[36] The term is used by Wollenweber (1913 a: 28).

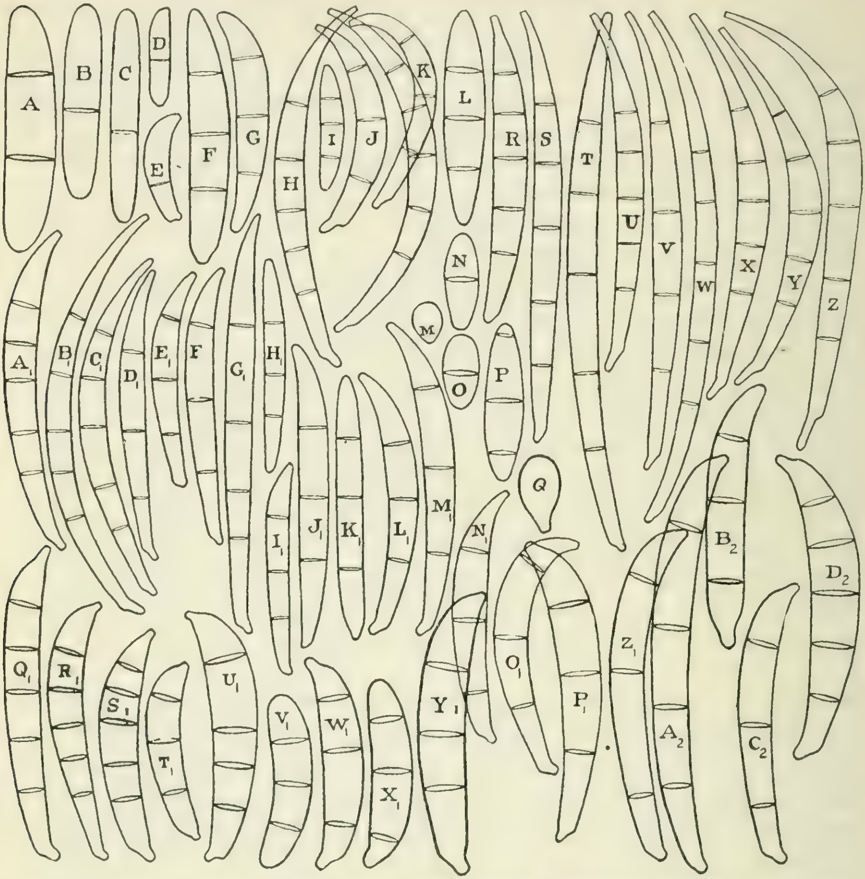

FIG. 1.— *Types of conidia of the species of Fusarium and Fusarium-like fungi found on potatoes.* A, *Ramularia Solani;* B, *R. eudidyma;* C, *R. Magnusiana;* D, *Fusarium affine;* E, *F. dimerum;* F, *F. cuneiforme;* G, *F. udum var Solani;* H, I, J, *F. ferruginosum;* K, *F. sanguineum var. pallidium;* L, M, N, O, P, *F. arthrosporioides;* Q, *F. sporotrichioides;* R, S, *F. arthrosporioides var. asporotrichius;* T, *F. anguioides;* U, *F. biforme;* V, *F. lucidum;* W, *F. subulatum;* X, *F. falcatum;* Y, *F. gibbosum;* Z, *F. caudatum var. Solani;* A_1, *F. bullatum;* B_1, *F. arcuosporum;* C_1, D_1, E_1, *F. truncatum;* F_1, *F. lutulatum;* G_1, H_1, *F. angustum;* I_1, J_1, K_1, *F. orthoceras;* L_1, *F. oxysporum var. resupinatum;* M_1, *F. oxysporum var. longius;* N_1, *F. oxysporum;* O_1, *F. sclerotioides;* P_1, *F. redolens var. Solani;* Q_1, *F. discolor;* R_1, *F. clavatum;* S_1, T_1, *F. subpallidum var. roseum;* U_1, V_1, *F. trichothecioides;* W_1, *F. discolor var. triseptatum;* X_1, Y_1, *F. Solani;* Z_1, A_2, *F. Martii;* B_2, *F. coeruleum;* C_2, *F. striatum;* D_2, *F. culmorum var. leteius. Magnification 1000 times*

DICHOTOMOUS KEY TO THE SPECIES OF FUSARIUM AND FUSARIUM-LIKE FUNGI OF POTATOES

	Page
a. Conidia[37] not typically dorsiventral, apex rounded, apedicellate. . . . . . RAMULARIA	264
b. Conidia typically one-septate	
c. Average diameter of one-septate conidia 4.9 μ. . . . . *R. eudidyma*	264
cc. Average diameter of one-septate conidia 3.9 μ. . . . . *R. Magnusiana*	265
bb. Conidia typically two-septate. . . . . *R. Solani*	267
aa. Conidia typically dorsiventral, apex more or less attenuate, mostly pedicellate FUSARIUM	125
b. Conidia typically one-septate, three or more septa never present. . Section **Dimerum**	125
c. Ventrally straight. . . . . *F. affine*	126
cc. Ventrally curved. . . . . *F. dimerum*	127
bb. Conidia typically three- or more septate	
c. Dorsiventrality slight. . . . . Section **Ventricosum**	128
d. Chlamydospores terminal only. . . . . *F. cuneiforme*	129
dd. Chlamydospores terminal and intercalary. *F. ventricosum*	128
cc. Dorsiventrality distinct	
d. Conidia apedicellate, true pionnotes present. . . Section **Eupionnotes.** *F. udum*	131
dd. Conidia pedicellate, true pionnotes absent	
e. Conidia with gradually attenuated, pointed apex	
f. Conidia prominently broader in the middle, apex long and narrow, most prominently pedicellate. . . . . Section **Gibbosum**	133
g. Dorsally more or less hyperbolic. . . . . *F. gibbosum*	133
gg. Dorsally more or less elliptic	
h. Aërial mycelium poorly developed, fruiting layers in form of a pseudopionnotes. . . . . *F falcatum*	135
hh. Aërial mycelium well developed	
i. Conidia typically five-septate. . . . . *F. falcatum* var. *fuscum*	138
ii. Conidia typically five- to seven-septate. . . . *F. caudatum* var. *Solani*	140
ff. Conidia of about equal diameter for a more or less considerable part of their length, apex not very long	
g. Conidia typically five-septate; typical color of conidial masses red, of substratum pink to orange and brown-red; no terminal chlamydospores	
h. Chlamydospores absent	
i. Microconidia typically absent. . . . . Section **Roseum**	142
j. Blue sclerotia present. . . . . *F. acuminatum*	142
jj. Blue sclerotia absent	
k. Conidia typically in pseudopionnotes; aërial mycelium weakly developed or absent. . . . . *F. metacroum*	143
kk. Conidia typically in distinct sporodochia; aërial mycelium well developed	
l. Sporodochia small, borne on aërial mycelium and without plectenchymic base. . . . . *F. subulatum*	147
ll. Sporodochia large, with plectenchymic base	
m. Six-septate conidia typically present. . . . . *F. effusum*	151
mm. Six-septate conidia typically absent	
n. Five-septate conidia dominant type. . . . . *F. lucidum*	157
nn. Three- and five-septate conidia about equally present, or three-septate dominant. . . . . *F. truncatum*	155

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[37] The term *conidia* is used here, in this key and elsewhere, in the sense of macroconidia.

Page

ii. Microconidia typically present, usually spindle-shaped, non- to three-septate........................Section **Arthrosporiella** 161

j. Six- to seven-septate conidia typically present, at least in pseudopionnotal stage

k. Distinct sporodochia present

l. Substratum and aërial mycelium near it typically of carmine hues, causal layer of aërial mycelium pink......*F. biforme* 166

ll. Substratum of a clay color, aërial mycelium white......... *F. diversisporum* 161

kk. No conspicuous sporodochia, fruiting layer on moist media commonly in form of a pseudopionnotes.......*F. anguioides* 169

jj. Six- to seven-septate conidia absent.........*F. arthrosporioides* 175

hh. Chlamydospores present, typically intercalary only

i. Microconidia present, pyriform............Section **Sporotrichiella** 183

F. sporotrichioides 183

ii. Microconidia absent or not pyriform.......Section **Ferruginosum** 186

j. Typical macroconidia of about equal diameter for a considerable length, or not prominently broader in the middle, in some cases approaching the subulatum type, much curved

k. Chlamydospores typically only in mycelium

l. Chlamydospores sparse, never in masses nor in long chains.. *F. arcuosporum* 186

ll. Chlamydospores typically abundant, commonly in masses of long chains and in clusters.............*F. ferruginosum* 190

kk. Chlamydospores also in conidia or in conidia only........ *F. sanguineum* 193

jj. Macroconidia typically noticeably broader at the middle, not very pointed at the apex, not much curved......*F. bullatum* 198

gg. Conidia typically three-septate; typical color of substratum vinaceous red to purplish vinaceous; terminal and intercalary chlamydospores present....................................Section **Elegans** 202

h. Conidiophores simple or only slightly branched

i. Average macroconidia 36 μ long....................*F. orthoceras* 202

ii. Average macroconidia 45.6 μ long...................*F. angustum* 203

hh. Conidiophores typically much branched

i. In plate cultures on neutral potato agar producing exposed and distinct pseudopionnotes

j. Color of the pseudopionnotes vinaceous red.*F. redolens* var. *Solani* 205

jj. Color of the pseudopionnotes vinaceous purple......*F. lutulatum* 209

ii. No exposed and distinct pseudopionnotes in potato agar plate cultures

j. Macroconidia typically somewhat broader toward apex........ *F. sclerotioides* 214

jj. Macroconidia typically not broader toward apex...*F. oxysporum* 220

ee. Conidia with more or less abruptly attenuated apex, rounded or papillate

f. Substratum typically (on glucose agar) carmine red. The color may be yellowish, but never gray, green, nor blue............Section **Discolor** 228

g. Conidia non- to three-septate, rounded at both ends, of common type, numerous (those of discolor type usually only few)..*F. trichothecioides* 229

gg. Discolor type of conidia common, and nearly the only type present

h. Conidial masses pale cream to pale pink in color, aërial mycelium well developed and nearly white....................*F. subpallidum* 230

Page

hh. Conidial masses of pale orange to dark chocolate-red in color, mycelium from nearly slightly pinkish to dense carmine red

i. Conidia much broadened toward apex................*F. clavatum* 234

ii. Conidia not or only slightly broadened toward apex

j. Average diameter of five-septate conidia from 4 to 4.4 μ....... *F. discolor* 236

jj. Average diameter of five-septate conidia from 5.8 to 6.8 μ...... *F. culmorum* 240

ff. Substratum typically (on a neutral or not strongly acid glucose agar) brownish gray, vinaceous red, vinaceous purple, or blue, but never carmine red.................................Section Martiella 244

g. Macroconidia of even diameter or slightly broader toward apex

h. Macroconidia comparatively long and narrow (from 7.4 to 9.3 times longer than broad)

i. Average three-septate conidia from 37 to 49 μ long.......*F. Martii* 244

ii. Average three-septate conidia from 34 to 35 μ long

j. Pseudopionnotes on an agar typically present, aërial mycelium poorly developed..............................*F. striatum* 255

jj. Pseudopionnotes on an agar typically absent, aërial mycelium well developed...............................*F. radicicola* 257

hh. Macroconidia comparatively short and broad (only about 5.5 times longer than broad)..................................*F. Solani* 251

gg. Macroconidia typically somewhat broader toward basal end........ *F. coeruleum* 260

DESCRIPTIONS OF SECTIONS, GENERA, SPECIES, AND VARIETIES

FUSARIUM Link

Link, Mag. Ges. nat. Freunde 3:10. 1824. Saccardo, Syll. Fung. 4:694. 1886. (Cf. Appel. O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. 8:60–61. 1910. Wollenweber, H. W., Phytopath. 3:24–50, 197–240, fig. 1, Pls. I and III. 1913; Ber. deut. Bot. Gesell. 31:17–34. 1913. Journ. Agr. Research 2:251–285. 1914.)

Hyphomycetes, with from hyaline to bright, but never plain gray nor black, conidia and mycelium; conidia sickle-shaped, septate (usually 3- or more septate), apically pointed, mostly pedicellate, not appendiculate, noncatenulate; conidia scattered over substratum, in pseudopionnotes or in sporodochia, the latter without or with from flat to wart-like plectenchymic substratum, and always without any differentiated enclosing or surrounding structures; conidiophores from simple to irregularly verticillate.

I. SECTION **DIMERUM** n. sec.

Conidia dorsiventral, 1-septate; chlamydospores may be present.

This section is proposed in order that Fusaria with 1-septate conidia may be included. The two species of this section are comparatively slow-growing fungi, with hyaline or nearly hyaline mycelium and from hyaline to orange-colored conidia. They appear to differ in many ways from typical Fusaria, but the differences are not sharp enough to warrant transferring them into another genus.

1. *Fusarium affine* Faut. et Lamb. (Figs. 1D and 2)

Fautrey, F., and Lambotte, E., Espèces nouvelles ou rares de la Côte-d'Or, Rev. Myc. Fr. **18**:68. 1896. Saccardo, Syll. Fung. **14**:1125. 1899.

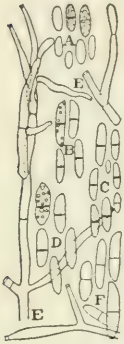

Fig. 2.—*Fusarium affine.* A, *Conidia from 35-days-old culture on slightly acidified potato agar;* B, *conidia from 7-days-old plate culture on hard potato agar;* C, *conidia from 74-days-old culture on red raspberry cane plug;* D, *conidia from 4-days-old culture on hard lima-bean agar;* E, *conidiophores from various media;* F, *conidia from 5-days-old culture on nutrient agar*

Conidia straight, somewhat dorsiventral near apex, apedicellate, typically 1–septate, 10.2 x 2.8 (9–11.4 x 2.6–3)μ, usually in a continuous smooth or slightly roughened, slimy layer, from hyaline to pale salmon-colored on a glucose agar; conidiophores from simple to sparingly branched, septate; mycelium hyaline; no chlamydospores.

Hab. In tubers and stems of *Solanum tuberosum*, in greenhouse soil, New York.

This organism was repeatedly isolated from various sources such as discolored fibrovascular bundles of potato tubers, from the base of wilted potato stems, and from soil. Its size is exactly the same as that given by Wollenweber (1913 c:229) for the conidial stage of *Mycosphaerella Solani* (E. et E.) Wr. Wollenweber's illustrations (Plate XXI, fig. N) are also much the same, and he considers *F. affine* Faut. et Lamb. as the conidial stage. The strain studied did not show any perfect form in culture, although it was grown for more than a year and on various media. This organism can be at once distinguished from all the other Fusaria by its minute, 1–septate, nearly straight conidia and its inconspicuous, slow growth.

For a fuller presentation of its septation and size the following detailed measurements are given:

On red raspberry cane plug, culture seventy-four days old:

Conidia: 0–septate, 45 per cent, 7 x 2.2 (3.5–9 x 1.7–2.6)μ
1–septate, 55 per cent, 9 x 2.6 (6–12 x 2–3)μ

On hard lima-bean agar, culture four days old:
Conidia: 0–septate, 40 per cent
1–septate, 60 per cent, 10.2 x 3 (9–11 x 2.8–3.8)μ

On nutrient agar, culture six days old:
Conidia: 0–septate, 10 per cent
1–septate, 90 per cent, 11.4 x 2.8 (8.7–13 x 2.4–3.5)μ

Average of the above measurements:
Conidia: 0–septate, 32 per cent, 7 x 2.2μ
1–septate, 68 per cent, 10.2 x 2.8μ

The size of the spores of *F. affine* as given by Saccardo (10–15 x 4μ) differs somewhat from the above, but the description otherwise is so much the same that the organisms can be considered identical.

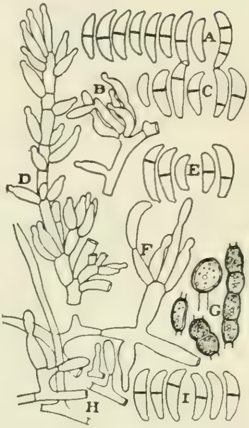

FIG. 3.—*Fusarium dimerum.* A, *Pseudopionnotal conidia from 70-days-old culture;* B, *conidiophore,* C, *pseudopionnotal conidia, from 19-days-old culture on slightly acidified hard potato agar;* D, *conidiophore from 70-days-old culture on potato stem plug;* E, *conidia,* F, *conidiophore, from 43-days-old culture on hard lima-bean agar with 2 per cent glucose;* G, *chlamydospores;* H, *conidiophores,* I, *conidia, from 21-days-old culture on hard lima-bean agar*

2. *Fusarium dimerum* Penz. (Figs. 1E and 3)

Penzig, O., Michelia 2:484. 1882. Saccardo, Syll. Fung. 4:704. 1886. Lindau, Rab. Krypt. Fl. Pilze 9:566. 1910. Appel and Wollenweber, Arb. K. biol. Anst. Land- u. Forstw. 8:37, text figs. 2 and 4. 1910.

Conidia lunar, somewhat pedicellate, typically 1–septate, 13 x 3.3 (12.5–13.5 x 3.3–3.4)μ, often also 0–septate, rarely 2– or 3–septate, borne singly on the mycelium or forming a more or less continuous slimy layer, from hyaline to cinnamon-buff on glucose agar; mycelium from hyaline to about the color of the conidial masses; chlamydospores intercalary, in mycelium.

Hab. On tubers and stems of *Solanum tuberosum* in Germany and in Minnesota (U. S. A.), and on fruits of *Citrus medica* in Italy.

The fungus is easily distinguished from all the other Fusaria by its lunar, minute, 1–septate conidia. It was isolated by the author only once, from a superficial dry rot of potato tuber received from St. Paul, Minnesota.

The measurements of the conidia in detail are as follows:

On hard potato agar, slightly acidified, culture eighteen days old:
Conidia: 0–septate, 3 per cent
1–septate, 97 per cent, 12.5 x 3.4 (10–18 x 2.9–4.1)μ

On hard lima-bean agar with 2 per cent glucose, culture forty-three days old:
Conidia: 0–septate, 12 per cent, 10 x 3.8μ
1–septate, 88 per cent, 12 x 3.3 (10–18 x 3–3.5)μ

On hard lima-bean agar, culture twenty-one days old:
Conidia: 0–septate, 27 per cent, 11.5 x 3.2 (9–14 x 2.9–3.5)μ
1–septate, 73 per cent, 12.5 x 3.3 (10–17 x 2.9–3.9)μ

Average of the above measurements:
Conidia: 0–septate, 14 per cent, 10.8 x 3.5μ
1–septate, 86 per cent, 12.8 x 3.3μ

The organism studied is so much the same as *F. dimerum* originally described by Penzig, that it is considered identical. A similar organism is also reported as occurring on potato in Germany (Appel and Wollenweber, 1910:37). The only peculiarity of the organism which either was not observed or was not present at all in the original *F. dimerum* is that the septum in the conidia of the organism studied is often and conspicuously acentric.

II. SECTION VENTRICOSUM Wr., Phytopath. 3:32

Conidia only slightly dorsiventral, somewhat wedge-shaped, broader toward base, apex somewhat rounded, apedicellate, typically 3–septate; no sporodochia; chlamydospores variable,[38] always present.

3. *Fusarium ventricosum* Ap. et Wr.

Wollenweber, H. W., Phytopath. 3:32, fig. 1, A and V. 1913.

Wollenweber describes this species as follows: "Conidia never formed in sporodochia, brownish-white to cream-colored, 3–septate, 29–37 x 5.75–7.5μ; conidiophores bostryx-like or irregularly branched, chlamydospores like those of the section Elegans. Wound parasite, ... found in Europe. Inhabits also *Beta vulgaris*."

The organism was neither isolated nor studied by the writer.

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[38] According to Wollenweber the chlamydospores are of Elegans type, that is, terminal and intercalary, 0– to 1– or more septate. In order to include *F. cuneiforme* it was necessary to alter the characterization of the chlamydospores in this section.

4. **Fusarium cuneiforme** n. sp. (Figs. 1 F and 4)

Conidia only slightly dorsiventral, more or less wedge-shaped, broader toward the base, with apex somewhat rounded, apedicellate, typically 3-septate, 34.7 x 5.6 (30–41 x 5.6–6)μ, often 0- to 2-septate, in false balls, from hyaline to cream-colored; no sporodochia; aërial mycelium hyaline, in a high tuft in center, and short, distinctly zonate, outside; chlamydospores from smooth to very distinctly warted, sometimes surrounded with a gelatinous capsule, terminal only, typically unicellular, 8.2 x 7.6 (7.6–8.5 x 7.3–8.1)μ.

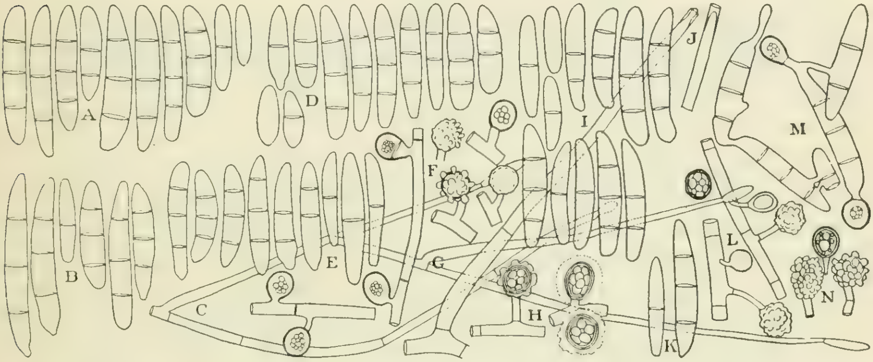

FIG. 4.—*Fusarium cuneiforme.* A, *Conidia from false balls of 16-days-old culture on slightly acidified hard potato agar;* B, *conidia from false balls from 10-days-old potato agar with 0.5 per cent glucose;* C, *conidiophores,* D, *conidia, from a thin layer of 73-days-old culture on red raspberry cane plug;* E, *conidia from confluent thin mass of false balls of 64-days-old culture on hard bean agar;* F, *terminal chlamydospores,* G, *conidiophore, from 34-days-old culture on hard lima-bean agar;* H, *terminal chlamydospores of 16-days-old culture on slightly acidified hard potato agar;* I, *conidia,* J, *tips of conidiophores showing beginning of production of new conidia, from 34-days-old culture on hard lima-bean agar;* K, *conidia,* L, *terminal chlamydospores, from 26-days-old corn agar;* M, *anastomosis, and chlamydospore producing conidia from 34-days-old culture on hard lima-bean agar;* N, *terminal chlamydospores from the same culture*

Hab. The fungus was isolated from soft rotted potato tubers received from Auburn, Alabama, and from Atlanta, New York, always in association with bacteria and other fungi.

Differs from *F. ventricosum* Ap. et Wr. mainly in typically 0-septate chlamydospores which are terminal only. There seems to be some difference also in the shape and size of the conidia, which here are somewhat more slender.

Latin description.—Conidiis parum dorsiventralibus, plus minusve cuneiformibus, deorsum latioribus, apice subrotundato, apedicellatis, typice 3–septatis, 34.7 x 5.6 (30–41 x 5.6–6)μ, 0–2–septatis, globis falsis, ex hyalino "cream color" (R); nullis sporodochiis; aerio mycelio hyalino, medio alte cristato, extra brevi et distincte zonato; chlamydosporis levibus vel maxime distincte verrucosis, interdum capsula gelatinosa cinctis, tantum terminalibus, typice unicellularibus, 8.2 x 7.6 (7.6–8.5 x 7.3–8.1)μ.

Hab. Fungus ex tuberibus mollibus putridisque Solani tuberosi ab Auburn, Alabama, et Atlanta, New York, Amer. bor. receptis, semper una cum bacteriis aliisque fungis, sejungebatur.

The measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture sixteen days old:
Conidia: 0–septate, rare, 16 x 4μ
1–septate, rare, 21 x 5.2μ
2–septate, 1 per cent, 24 x 5.6 (20–32 x 5.2–5.8)μ
3–septate, 99 per cent, 35.5 x 6 (24–44 x 4.7–7.5)μ

On red raspberry cane plug, culture seventy-three days old:
Conidia: 0–septate, 12 per cent, 14 x 5.9 (6.5–17 x 5–6.5)μ
1–septate, 34 per cent, 19 x 6 (15–27 x 4.8–7)μ
2–septate, 11 per cent, 33 x 5.9 (20–40 x 5.2–6.5)μ
3–septate, 43 per cent, 33 x 5.9 (20–40 x 5.2–6.5)μ

On hard potato agar, culture twenty-five days old:
Conidia: 1–septate, rare
3–septate, 100 per cent, 35 x 5.8 (24–48 x 5–6.5)μ

On hard lima-bean agar with 2 per cent glucose, culture sixty-four days old:
Conidia: 0–septate, 4 per cent
1–septate, 64 per cent, 25 x 4.9 (12–41 x 3.5–6.5)μ
2–septate, 22 per cent, 28 x 5.5 (23–32 x 4.8–5.9)μ
3–septate, 10 per cent, 30 x 5.6 (24–38 x 4.8–6.2)μ

On hard lima-bean agar, culture thirty-four days old, advanced part of colony growth:
Conidia: 0–septate, rare
1–septate, 17 per cent
2–septate, 7 per cent
3–septate, 76 per cent, 31.5 x 6 (27–41 x 5.2–7.6)μ

On same culture as above but from an old part of the colony growth:
3–septate, 70 per cent, 41 x 6 (36–46 x 5.2–7.6)μ

Average of the above measurements:

Conidia: 0–septate, 3 per cent, 15 x 5μ
1–septate, 23 per cent, 21.3 x 5.4μ
2–septate, 8 per cent, 26 x 5.45μ
3–septate, 66 per cent, 34.7 x 5.9μ

III. SECTION EUPIONNOTES Wr., Phytopath. 3:38, 206, 219

Conidia dorsiventral, apedicellate, nearly cylindrical for the largest part or slightly broader toward apex, typically 3–septate, in true pionnotes; terminal and intercalary chlamydospores present. Differs from all the other sections of Fusaria by true pionnotal fruiting form.

5. *Fusarium udum* (Berk.) Wr.

Syn. *Fusisporium udum* Berk., Ann. Mag. Nat. Hist. 6:438, Pl. XIV, fig. 28. 1841.
Pionnotes uda (Berk.) Sacc., Syll. Fung. 4:726. 1886.

Cf. Wollenweber, Phytopath. 3:38 (footnote), 219 (key), Pl. XXI, figs. R and S.

According to Wollenweber's data and figures, the conidia are dorsiventral with somewhat rounded apex, apedicellate, typically 3–septate, about 33–45 x 3.5–4μ, sometimes 4– or 5–septate; terminal chlamydospores present.

Hab. On cut surfaces of oak, elm, and other trees, also on *Solanum tuberosum*, on tulip bulbs, and in the soil.

6. *Fusarium udum* (Berk.) Wr. var. **Solani** n. var. (Figs. 1G and 5)

Conidia dorsiventral, usually somewhat broader toward the slightly rounded apex, apedicellate, typically 3–septate, 30.2 x 4.27 (25–34 x 4–4.5)μ, non– to two–septate very rare when mature, 4– and 5–septate rare, of from light vinaceous cinnamon to orange-cinnamon hues on agars rich in glucose; chlamydospores usually found only in old cultures, terminal and intercalary, in conidia, in the tips of sterigmata, and in mycelium, often of dense orange color, 0–septate, 6 x 5.5μ; aërial mycelium present only near margin of colony growth, very loose, short, hyaline; substratum colorless or approaching the color of the conidia.

Hab. On rotted tubers of *Solanum tuberosum*, together with *Ramularia Solani*, Long Island, New York.

Differs from *F. udum* (Berk.) Wr. in that the conidia are shorter and thicker, and somewhat broader toward the apex.

The following measurements were taken:

On slightly acidified hard potato agar, culture eight days old:

Conidia: 0– and 1–septate, only when young
3–septate, 100 per cent, 33 x 4.25 (28–39 x 3.5–4.7)μ

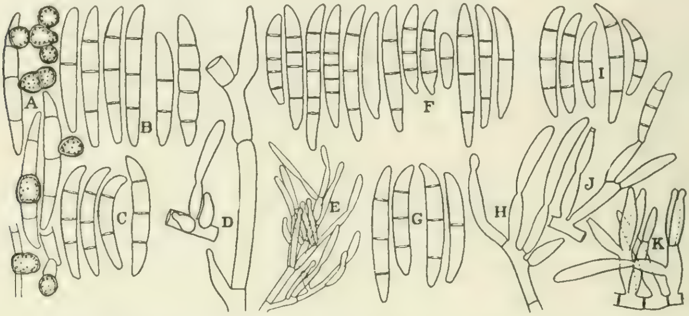

Fig. 5.— *Fusarium udum var. Solani.* A, *Different forms of chlamydospores from 85-days-old culture on hard potato agar with 5 per cent glucose;* B, *conidia from 9-days-old culture on hard lima-bean agar;* C, *conidia,* D, *conidiophores, from 49-days-old culture on rye straw;* E, *conidiophores (magnified 250 times) from 8-days-old culture on slightly acidified hard potato agar;* F, *conidia from 72-days-old culture on potato tuber plug;* G, *conidia,* H, *conidiophores, from 8-days-old culture on slightly acidified hard potato agar;* I, *conidia,* J, K, *conidiophores, from 74-days-old culture on red raspberry cane plug*

On red raspberry cane plug, culture seventy-four days old:

(1) From an advanced part of colony growth

Conidia: 1–septate, rare
2–septate, 10 per cent, 22 x 3.8 (19.5–24 x 3.3–4.1)μ
3–septate, 90 per cent, 25 x 4 (22–31 x 3.9–4.6)μ

(2) From an old part of colony growth

Conidia: 3–septate, 100 per cent, 28 x 4μ dominant type
4–septate, rare

On potato tuber plug, culture seventy-two days old:

Conidia: 0–septate, rare, young
1–septate, 5 per cent, mostly young
2–septate, 2 per cent, mostly young
3–septate, 86 per cent, 31 x 4.4 (23–40 x 3.9–4.7)μ
4–septate, 7 per cent, 32 x 4.4 (28–40 x 4.1–4.7)μ
5–septate, rare, 35 x 4.4 (30–40 x 4–4.6)μ

On hard lima-bean agar, culture four days old:

Conidia: 1–septate, 1 per cent, young
2–septate, 4 per cent, 31 x 4.4
3–septate, 95 per cent, 34 x 4.5 (25–40 x 4.1–4.9)μ
4–septate, rare, 40 x 5.1 (only a few measured)

Average of the above measurements:

Conidia: 0–septate, rare to none
1–septate, 1.5 per cent, (usually immature)
2–septate, 4 per cent
3–septate, 93 per cent, 30.2 x 4.27μ
4–septate, 1.5 per cent, 36 x 4.75μ
5–septate, rare to none

IV. SECTION GIBBOSUM Wr., Phytopath. 3:31, fig. 1, L and M

Conidia with from hyperbolic or parabolic to elliptic dorsal curve, conspicuously broader in the middle, with more or less long, narrow apex, prominently pedicellate, mostly 5–septate; intercalary chlamydospores always present; color of substratum and conidial mass typically from pale buff to cinnamon and sepia; mycelium from hyaline to brown.

7. *Fusarium gibbosum* Ap. et Wr. (Figs. 1Y and 6)

Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. 8:185–190, text fig. 10, C and D. 1910. Wollenweber, H. W., Phytopath. 3:32, fig. 1, M. 1913.

Conidia often with hyperbolic dorsal curve, conspicuously broader in the middle, with long, narrow apex, prominently pedicellate, typically 5-septate, 41.6 x 4.6 (40–46 x 4.4–4.7)μ, sometimes also 0– to 7–septate, in minute nonconverging sporodochia or spreading over mycelium singly, from hyaline to light pinkish cinnamon in color; chlamydospores intercalary, always present; aërial mycelium typically present, short, fine, medium loose; colony faintly zonate; substratum on potato agar rich in glucose, from pale flesh to cinnamon in color.

Hab. On stems and tubers of *Solanum tuberosum* in Germany, rare.

The organism was not isolated by the writer, but a culture of it was carefully examined on several different media with the following results in regard to spore septation and size:

On slightly acidified hard potato agar, culture twenty-four days old:

Conidia: 3–septate, 5 per cent
4–septate, 15 per cent, 32–4.1μ
5–septate, 80 per cent, 43.2 x 4.6 (38–50 x 4.3–4.9)μ

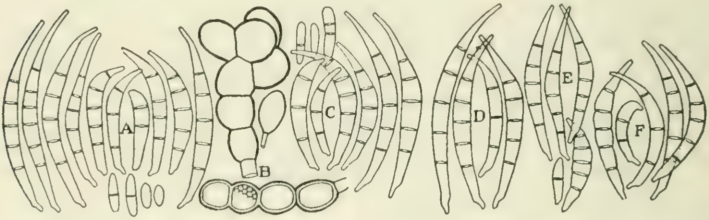

Fig. 6.—*Fusarium gibbosum.* a, *Pseudopionnotal conidia from 10-days-old culture on hard lima-bean agar;* b, *intercalary chlamydospores,* c, *conidia, from 71-days-old culture on red raspberry cane plug;* d, *pseudopionnotal conidia from 118-days-old culture on hard lima-bean agar with 2 per cent glucose;* e, *conidia from 42-days-old culture on potato stem plug;* f, *pseudopionnotal conidia from 24-days-old culture on slightly acidified hard potato agar*

On red raspberry cane plug, culture seventy-one days old:

Conidia: 3–septate, 5 per cent
4–septate, 5 per cent
5–septate, 90 per cent, 40 x 4.6 (33–46 x 4.2–5.2)μ
6–septate, rare

On hard lima-bean agar with 2 per cent glucose, culture one hundred and eighteen days old:

Conidia: 4–septate, 1 per cent
5–septate, 94 per cent, 37 x 4.6 (26–44 x 3.9–4.9)μ
6–septate, 3 per cent, 42 x 4.7μ
7–septate, 2 per cent

On hard lima-bean agar, culture ten days old:

Conidia: 0–septate, 1.5 per cent, 7.5 x 3.2 μ
1–septate, 4 per cent, 10 x 3.2 (7–17 x 2.7–3.5)μ
2–septate, very rare
3–septate, 4 per cent, 27 x 4.1 (19–41 x 3.5–4.1)μ
4–septate, 3 per cent
5–septate, 86 per cent, 46 x 4.4 (35–58 x 4–4.8)μ
6–septate, 1.5 per cent, 52 x 4.5 (50–60 x 4.3–4.8)μ

Average of the above measurements:

Conidia: 0–septate, 0.5 per cent, 7.5 x 3.2μ
1–septate, 1 per cent, 10 x 3.2μ
2–septate, very rare
3–septate, 4 per cent, 27 x 4.1μ
4–septate, 6 per cent, 27 x 4.1μ
5–septate, 87 per cent, 41.6 x 4.6μ
6– and 7–septate, 1.5 per cent, 47 x 4.6μ

The averages of Appel and Wollenweber's (1910:189–190) measurements are as follows:

Conidia: 3–septate, 3 per cent, 25 x 4.5μ
4–septate, 2 per cent, 29 x 4.5μ
5–septate, 59 per cent, 42 x 4.4μ
6–septate, 23 per cent, 49 x 4.6μ
7–septate, 13 per cent, 49 x 4.7μ

There is a notable difference between the data presented by the writer and those of Wollenweber only in the proportion of 6– and 7–septate conidia, the size of 5– and of 6– and 7–septate conidia being very much the same.

8. *Fusarium falcatum* Ap. et Wr. (Figs. 1x and 7)

Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. 8:175–185; Pl. II, figs. 100 to 110; Pl. III, fig. 9; text fig. 10, A. 1910.

Syn. *Fusarium vasinfectum* var. *Pisi* Schikorra, Arb. K. biol. Anst. Land- u. Forstw. 4:157, Pl. VII, 1906; not *F. vasinfectum* var. *Pisi* van Hall, Ber. deut. Bot. Gesell. 21:4, pl. 4, 1903. Wollenweber, Phytopath. 3:31, fig. 1, L, 1913.

Conidia often with parabolic dorsal curve, conspicuously broader in the middle, with long and narrow apex, prominently pedicellate, typically 5–septate, 49.1 x 4.6 (43–54.5 x 4.5–4.7)μ, often 3- to 7–septate, 0- to 2- and 8–septate very rare, in minute sporodochia more or less converging into pseudopionnotes, from buff-pink to cinnamon on hard potato agar rich in glucose; intercalary chlamydospores always present; aërial mycelium very poorly developed or not developed at all, leaving slimy layer exposed; substratum about the same color as the spores.

Hab. Often on *Pisum sativum*, seldom on underground part of stem of *Solanum tuberosum*, in Germany, and cause of fruit rot of *Solanum lycopersicum* in Germany and in the United States.

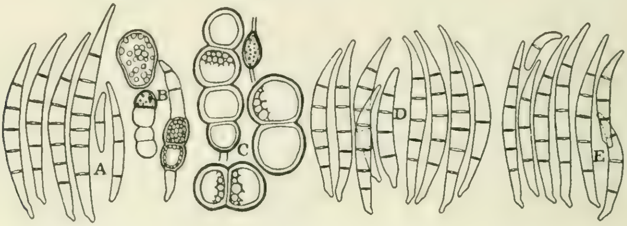

Fig. 7.—*Fusarium falcatum.* A, *Pseudopionnotal conidia,* B, *chlamydospores in mycelium and in spore, from 119-days-old culture on hard lima-bean agar with 2 per cent glucose;* C, *chlamydospores,* D, *pseudopionnotal conidia, from 71-days-old culture on red raspberry cane plug;* E, *pseudopionnotal conidia from 42-days-old culture on potato stem plug*

Differs from *F. gibbosum* mainly in having parabolic dorsal curve and typically exposed pseudopionnotes; also in considerably longer conidia.

The organism was not isolated by the writer, but the culture was obtained through the courtesy of Dr. Wollenweber. The writer's cultural observations in regard to septation and size of spores of this organism on various media are as follows:

On slightly acidified hard potato agar, culture twenty-four days old:

Conidia: 3–septate, 3 per cent, 28–42 x 3.5–4μ
4–septate, 9 per cent, 33–42 x 3.5–4μ } (only a few measured)
5–septate, 77 per cent, 50 x 4.5 (43–58 x 4–5.3)μ
6–septate, 10 per cent, 54 x 5 (50–60 x 4.8–5.3)μ
7–septate, 1 per cent, about 60–70 x 4.8–5.3μ

On red raspberry cane plug, culture seventy-one days old:

Conidia: 3–septate, 2 per cent
4–septate, 3 per cent
5–septate, 90 per cent, 43 x 4.6 (36–49 x 4–5.2)μ
6–septate, 5 per cent, 46 x 4.8 (43–53 x 4.3–5.2)μ

On hard lima-bean agar with 2 per cent glucose, culture one hundred and nineteen days old:

Conidia: 1–septate, 2.5 per cent, 15 x 2.7μ (only two measured)
3–septate, 3 per cent, 30 x 3.6 (10–44 x 3–4.7)μ
4–septate, 5 per cent
5–septate, 81 per cent, 49 x 4.7 (42–55 x 4.3–5.2)μ
6–septate, 6 per cent, 51 x 4.9 (45–56 x 4.6–5.2)μ
7–septate, 2.5 per cent, 52 x 5.2 (47–58 x 5.2)μ
8–septate, exceptionally rare, 58 x 5.4 (only one measured)

On hard lima-bean agar, culture eleven days old:

Conidia: 0–septate, rare
2–septate, 2 per cent
3–septate, 30 per cent, 30 x 3.6 (20–40 x 3–4.3)μ
4–septate, 5 per cent
5–septate, 62 per cent, 54.5 x 4.5 (43–65 x 3.5–5.2)μ
6–septate, 1 per cent, 64 x 4.8 (59–70 x 4.6–5.3)μ
7–septate, rare, same as 6–septate

Average of the above measurements:

Conidia: 0–septate, very rare
1–septate, 0.5 per cent
2–septate, 0.5 per cent, 15 x 2.7μ
3–septate, 9.5 per cent, 30 x 3.6μ
4–septate, 5.5 per cent
5–septate, 77.5 per cent, 49.1 x 4.56μ
6–septate, 5.5 per cent, 54 x 4.9μ
7–septate, 1 per cent, 58 x 5.1μ
8–septate, very rare, 58 x 5.4μ

Averages of Appel and Wollenweber's (1910:184) measurements are as follows:

Conidia: 5–septate, 46 x 4.7μ
6–septate, 49 x 4.4μ

The measurements of the writer show some deviation from these, but the deviation is small and can be explained entirely by the fact that the writer's measurements were taken from much younger cultures, which usually yield somewhat longer conidia than those produced in old cultures.

9. *Fusarium falcatum* Ap. et Wr. var. **fuscum** n. var. (Fig. 8; Pl. vii, fig. 8)

Conidia with from ellipsoidal to parabolic dorsal curve, conspicuously broader in the middle, prominently pedicellate, typically 5–septate, 45 x 4.6 (40–51 x 4.4–4.7)μ, 3– and 4–septate ones also present, 6– and 7–septate rare, 8–septate very rare, typically in conspicuous plectenchymic or aplectenchymic sporodochia, from light buff and honey yellow to buckthorn and cacao brown, on potato agar rich in glucose; chlamydospores intercalary, always present, sometimes by their abundance and color making the entire medium and the aërial mycelium of a dark brown color; aërial mycelium always present, high, from fine to medium fine, more or less loose, hyaline at first, later becoming from tawny olive to brown; color of substratum on agars from hyaline to that of the spores.

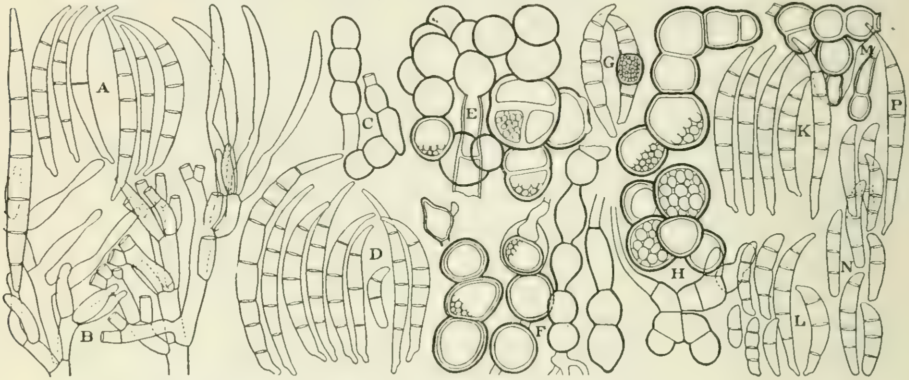

Fig. 8.— *Fusarium falcatum var. fuscum.* A, *Pseudopionnotal conidia,* B, *conidiophores, from 4-days-old culture on hard potato agar;* C, *intercalary chlamydospores,* D, *pseudopionnotal conidia, from 10-days-old culture on hard bean agar;* E, *cluster of intercalary chlamydospores from 99-days-old culture on potato tuber plug;* F, *intercalary chlamydospores in long chains,* G, *conidia with and without chlamydospores, from 176-days-old culture on corn agar;* H, *young and old chlamydospores,* K, *sporodochial,* L, *aërial, conidia, from 79-days-old culture on red raspberry cane plug;* M, *intercalary and terminal chlamydospores,* N, *aërial conidia, from 50-days-old culture on rye straw;* P, *typical conidium from a large sporodochium of 29-days-old culture on hard oat agar*

Hab. On rotted tubers of *Solanum tuberosum*, New York State.

Differs from *F. falcatum* mainly by large sporodochia, more profuse chlamydospore production, well-developed aërial mycelium, and typical absence of pseudopionnotes.

The organism was isolated only once, from a potato tuber slightly rotted near the stem end, which was received from a potato grower in New York. The measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture four days old:

Conidia: 0– and 1–septate, few, very young
3–septate, 4 per cent, 38 x 4.3 (35–42 x 4.1–4.7)μ
4–septate, 6 per cent, 41.6 x 4.4 (35–44 x 4.1–4.7)μ
5–septate, 88 per cent, 45.5 x 4.6 (40–49 x 4.1–4.8)μ
6– and 7–septate, 2 per cent, 50–80 x 5.5μ (only a few measured)
The largest 5–septate conidium observed measured 67 x 5.8μ

On red raspberry cane plug, culture seventy-nine days old:

Conidia: 3–septate, 1 per cent
4–septate, 4 per cent
5–septate, 95 per cent, 40 x 4.7 (31–48 x 4.3–5.2)μ

On hard lima-bean agar, culture ten days old:

Conidia: 3–septate, 3 per cent, 34 x 4.4 (19–40 x 3.1–4.7)μ
4–septate, 6 per cent
5–septate, 91 per cent, 45 x 4.4 (36–61 x 4–4.8)μ

On hard oat agar, culture twenty-nine days old, from a sporodochium about 3 millimeters in diameter:

Conidia: 3–septate, 2 per cent
4–septate, 5 per cent
5–septate, 93 per cent, 51 x 4.6 (45–53 x 4.1–4.9)μ

Average of the above measurements:

Conidia: 3–septate, 2.5 per cent, 36 x 4.3μ
4–septate, 5 per cent, 41.6 x 4.4μ
5–septate, 92 per cent, 45 x 4.6μ
6– and 7–septate, 0.5 per cent

10. *Fusarium caudatum* Wr. var. **Solani** n. var. (Figs. 1z and 9; Pl. iv, fig. 7; Pl. vi, fig. 3)

Conidia with from parabolic to ellipsoid dorsal curve, conspicuously broader at the middle, with very long, narrow, whiplike apex, prominently pedicellate, typically 5– to 7–septate; measuring on the average, 5–septate, 48 x 4.6 (40–55 x 4.3–4.7)μ, 7–septate, 64.7 x 4.6 (57–69 x 4.4–4.8)μ; rarely in pseudopionnotes, typically in small aplectenchymic sporodochia, tinted from cream-buff to cinnamon, clay, and Saccardo's amber in a plate culture on a potato hard agar rich in glucose; chlamydospores intercalary, always present in greater or less abundance; aërial mycelium very well developed, high, uniform, medium dense, from hyaline when young to sepia in old cultures, mostly from brownish to dresden brown; substratum, on potato agar rich in glucose, from pinkish buff when young to ochraceous tawny and snuff brown shaded to sepia in very old cultures.

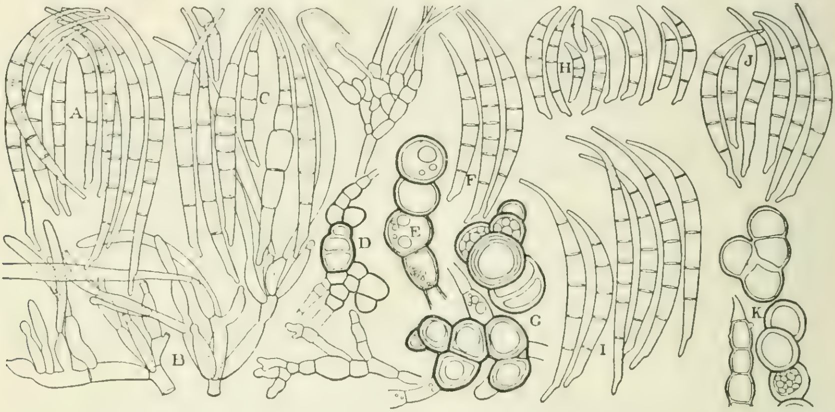

Fig. 9.— *Fusarium caudatum var. Solani.* A, *Pseudopionnotal conidia,* B, *conidiophores, from 9-days-old culture on slightly acidified hard potato agar;* C, *pseudopionnotal conidia from 38-days-old culture on potato stem plug;* D *and* E, *chlamydospores from 50-days-old culture on red raspberry cane plug (magnification 250 times);* F, *conidia from aërial sporodochium of 118-days-old culture on hard lima-bean agar with 2 per cent glucose;* G, *cluster of chlamydospores,* H, *aërial conidia, from 50-days-old culture on rye straw;* I, *sporodochial conidia in oozing drop of liquid from 9-days-old culture on hard lima-bean agar;* J, *aërial conidia,* K, *intercalary chlamydospores, from 69-days-old culture on red raspberry cane plug*

Hab. On superficial dry-rot spots on tubers of *Solanum tuberosum*, Atlanta, New York.

Differs from *F. caudatum* Wr. (see Wollenweber 1914:262–263, Pl. XVI, fig. M) mainly by broader conidia, which in the latter organism average only from 3 to 4.5μ in diameter.

The organism was twice isolated, in 1912 and in 1913, from superficial dry rot of potato tubers collected at Atlanta, New York. The measurements of conidia on different media are as follows:

On red raspberry cane plug, culture sixty-nine days old:
Conidia: 3–septate, 10 per cent, 30 x 4 (26–35 x 3.5–4.5)μ
4–septate, 5 per cent, 34 x 4.4 (32–40 x 4–4.8)μ
5–septate, 85 per cent, 40 x 4.8 (33–55 x 4–5.4)μ

On slightly acidified hard potato agar, culture nine days old:
Conidia: 3–septate, 1 per cent, about 38 x 4μ (only a few measured)
4–septate, 8 per cent, about 40 x 4.2μ (only a few measured)
5–septate, 43 per cent, 55 x 4.3 (38–69 x 3.5–5.9)μ
6–septate, 27 per cent, 65 x 4.4 (55–84 x 4.1–5.3)μ
7–septate, 21 per cent, 69 x 4.4 (60–85 x 4.1–5.9)μ
8–septate, very rare

On hard lima-bean agar with 2 per cent glucose, culture one hundred and eighteen days old:
Conidia: 4–septate, 1 per cent
5–septate, 29 per cent, 46 x 4.7 (43–59 x 4.3–5.2)μ
6–septate, 32 per cent, 53 x 4.8 (48–60 x 4.3–5)μ
7–septate, 37 per cent, 57 x 4.8 (50–62 x 4.7–5)μ
8–septate, 1 per cent, 61 x 5μ (only one measured)

On hard lima-bean agar, culture nine days old:
Conidia: 5–septate, 55 per cent, 52 x 4.5 (45–61 x 4–4.8)μ
6–septate, 35 per cent, 65 x 4.7 (55–71 x 4.3–4.8)μ
7–septate, 10 per cent, 68 x 4.7 (58–78 x 4.3–4.8)μ

Average of the above measurements:
Conidia: 3–septate, 3 per cent, 34 x 4μ
4–septate, 3.5 per cent, 37 x 4μ
5–septate, 53 per cent, 48 x 4.6μ
6–septate, 23.5 per cent, 58 x 4.6μ
7–septate, 17 per cent, 64.7 x 4.6μ
8–septate, rare, 61 x 5μ (only one measured)

V. SECTION ROSEUM Wr. (emended), Phytopath. 3:32, fig. 1 N, 1913

Conidia broad ellipsoid, typically of an even diameter for a considerable part of their length, comparatively narrow (from 3.6 to 4.3μ in average diameter), always very gradually attenuate toward both ends, conidia of all fruiting forms of the same shape and type; true chlamydospores always absent; on agars rich in glucose, from honey yellow and morocco red to Eugenia red, sometimes nearly hyaline.

11. *Fusarium acuminatum* Ell. et Ev. emend. Wr.

Cf. Wollenweber, H. W., Journ. Agr. Research 2:269–270, Pl. XVI, fig. G. 1914. *Fusarium acuminatum* Ell. et Ev., Proc. Acad. Sci. Phila. 1895:441. Saccardo, Syll. Fung. 14:1125–1126. 1899. Wollenweber's diagnosis (page 269 of reference cited) is as follows:

" Conidia, scattered, in sporodochia or in pionnotes, orange in mass. Conidia average as follows: 5–septate, 40 to 70 by 3 to 4.5μ; 4–septate (less common), 30 to 60 by 3 to 4.5μ; 3–septate, 20 to 45 by 2.75 to 4.25μ. Conidia of 0–, 1–, 2–, 6–, and 7–septations are occasionally found. Subnormal small conidia may be mistaken for conidia of the section Discolor, but normal sporodochia develop on repeatedly whorl-like branched conidiophores, giving the characteristic conidia of the section Roseum. The conidia show in side view hyperbolic or parabolic curves, in contrast to *Fusarium metacroum* App. and Wollenw., the conidia of which are as a rule more nearly straight. Blue globose sclerotia, 50–70μ thick, occur and form a striking contrast to the carmine plectenchymatic thallus on starchy media, such as steamed potato tubers. Both blue and carmine are basic modifications of the fungus, while yellow (on rice) is the acid one, turning blue to purple violet with the addition of an alkali.

" Habitat. Occurs on partly decayed plants, especially on stems, roots, and tubers, also on fruits. Found on Solanum, Ipomoea, Fagus (beech nuts), and *Impatiens balsamina* in the United States of America."

Ellis and Everhart's description is incomplete, but Wollenweber says (on page 270 of reference cited) that he " found this fungus so widely distributed on potato stems in the New England States that he feels justified in identifying it as *Fusarium acuminatum*." The writer did not study this fungus.

12. *Fusarium metacroum* Ap. et Wr. (Fig. 10; Pl. vii. fig. 5)

Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. 8:132–141; Pl. i, figs. 111 to 118; Pl. iii, fig. 8. 1910.

Conidia broad, ellipsoid, more or less pointed at apex, seldom prominently pedicellate, typically 5–septate, 53 x 4.1 (43–65 x 3.8–4.3)μ, often 3– or 4–septate, seldom 0– to 2–, rarely 6–, exceptionally up to 12–septate, in minute, more or less converging, sporodochia forming exposed pseudopionnotes, from corinthian red to clay color, typically from dragon's-blood red to brick red; chlamydospores absent; aërial mycelium typically absent; substratum from madder brown and brazil red to russet color; conidia often densely granulate with indistinct septation.

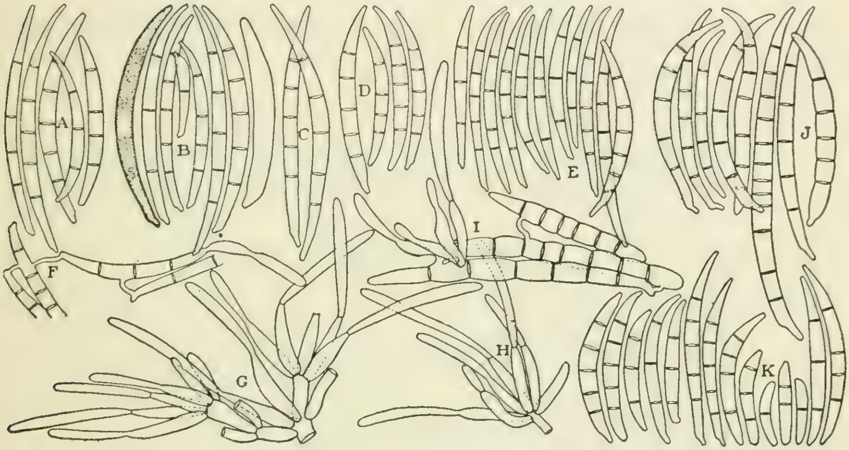

Fig. 10.— *Fusarium metacroum. Pseudopionnotal conidia:* a, *from 65-days-old culture on rye grain;* b, *from 10-days-old culture on hard lima-bean agar;* c, *from 8-days-old culture on hard lima-bean agar with 2 per cent glucose;* d, *from 113-days-old culture on potato stem plug;* e *and* f, *from 23-days-old culture on red raspberry cane plug;* i *and* j, *from 11-days-old culture on slightly acidified hard potato agar;* k, *from 66-days-old culture on rye grain.* g *and* h, *Conidiophores from 23-days-old culture on red raspberry cane plug*

Hab. On grains of *Triticum vulgaris* in Germany and on rotted tubers of *Solanum tuberosum* in New York State.

The organism was isolated, together with *F. diversisporum*, from a rotted tuber from Long Island. The two fungi were growing together, and in the original culture the mixture appeared to be a pink Fusarium. The fungi were separated by dilution and remained very distinct from each other. *F. metacroum* isolated by the writer is in all respects identical with the originally described organism.

The measurements of the conidia from the original culture when grown on various media are as follows:

On red raspberry cane plug, culture twenty-three days old:

Conidia: 3–septate, 42 per cent, 46 x 3.2 (35–56 x 2.6–4.1)μ
4–septate, 28 per cent, 50 x 3.5 (40–62 x 2.9–4.1)μ
5–septate, 30 per cent, 50 x 3.8 (45–63 x 3.3–4.3)μ

On hard lima-bean agar with 2 per cent glucose, culture eleven days old:

Conidia: 3–septate, 8 per cent
4–septate, 7 per cent
5–septate, 75 per cent, 54 x 4.1 (35–62 x 3.5–5.7)μ
6–septate and more, 10 per cent, the largest spore 12–septate, 91 x 5.3μ

On slightly acidified hard potato agar, culture eight days old:

Conidia: 3–septate, 15 per cent
4–septate, 15 per cent
5–septate, 70 per cent, 65 x 4.2 (55–73 x 3.8–4.8)μ

On potato tuber plug, culture ninety-eight days old:

Conidia: 1–septate, 5 per cent, 19 x 3 (14–21 x 2.7–3.5)μ
2–septate, few
3–septate, 10 per cent, 36 x 3.8 (28–43 x 3.5–4.2)μ
4–septate, 30 per cent, 46 x 3.9 (36–51 x 3.5–4.7)μ
5–septate, 55 per cent, 50 x 4 (42–63 x 3.5–4.4)μ

On potato stem plug, culture one hundred and thirteen days old:

Conidia: 3–septate, 25 per cent, 36 x 3.7 (33–39 x 3.4–3.9)μ
4–septate, 20 per cent, about 39 x 4μ (only a few measured)
5–septate, 55 per cent, 43 x 4 (38–47 x 3.5–4.2)μ, the longest 5–septate, 63 x 3.85μ

On whole steamed potato tuber, culture thirty-eight days old:

Conidia: 3–septate, 40 per cent, 42 x 3.9 (37–53 x 3.5–4.1)μ
4–septate, 35 per cent, 45 x 4.1μ
5–septate, 25 per cent, 54 x 4.3 (47–63 x 3.7–4.8)μ

On rye grain, culture sixty-five days old:

(1) From a slimy heap of conidia 1 millimeter in diameter
Conidia: 3–septate, 27 per cent, 43 x 3.8 (31–49 x 2.9–4.7)μ
4–septate, 11 per cent, 44 x 4.1 (40–63 x 2.6–4.8)μ
5–septate, 62 per cent, 54.5 x 4.1 (43–67 x 3–5.2)μ

(2) From a minute, semi-dry fleck of conidia, culture sixty-six days old
Conidia: 0–septate, 2 per cent
1–septate, 3 per cent, 20 x 3.5 }
2–septate, 3 per cent, 23 x 3.9 } (only a few measured)
3–septate, 65 per cent, 32 x 3.9 (22–47 x 3.5–4.4)μ
4–septate, 12 per cent, 43 x 4.1 (38–48 x 3.5–4.4)μ
5–septate, 15 per cent, 45 x 4.3 (38–48 x 3.5–4.7)μ, the largest 53 x 5.25μ

On hard lima-bean agar, culture ten days old:
Conidia: 3–septate, 6 per cent, 48 x 3.7μ (only three measured)
4–septate, 4 per cent
5–septate, 90 per cent, 61 x 3.9 (43–68 x 3.5–4.1)μ

Average of the above measurements:
Conidia: 0– to 2–septate, rare
3–septate, 26 per cent, 40.4 x 3.7μ
4–septate, 19 per cent
5–septate, 54 per cent, 53 x 4.1μ
6– and 7–septate, 1 per cent

The averages of Appel and Wollenweber's measurements are as follows:
Conidia: 3–septate, 9 per cent, 39 x 3.9μ
4–septate, 14 per cent, 43 x 4.2μ
5–septate, 76 per cent, 53 x 4.3μ
6–septate, 1 per cent, 63 x 4.6μ

13. *Fusarium metacroum* Ap. et Wr. var. **minus** n. var. (Fig. 11)

Conidial type as in *F. metacroum*, 5–septate conidia 54 x 3.6 (46–60 x 3.4–3.9)μ.

Hab. On stem of *Solanum tuberosum*, New York State.

Differs from *F. metacroum* by narrower conidia, often distinct plectenchymic substratum, and swellings in hyphæ very similar to true chlamydospores.

The organism was isolated only once, from a half-dead stem of potato plant at Atlanta, New York, from a pseudopionnotal spore mass. The measurements of the conidia produced by the organism on various media are as follows:

On red raspberry cane plug, culture thirty-one days old:

Conidia: 1–septate, 0.5 per cent, 20 x 2.5μ
2–septate, very few
3–septate, 46.5 per cent, 43 x 2.8 (23–53 x 2.3–3.5)μ
4–septate, 31 per cent, 48 x 3.2 (40–60 x 2.3–3.5)μ
5–septate, 22 per cent, 52 x 3.4 (42–60 x 2.9–4)μ
6–septate, rare, 61 x 3.4μ (only one measured)

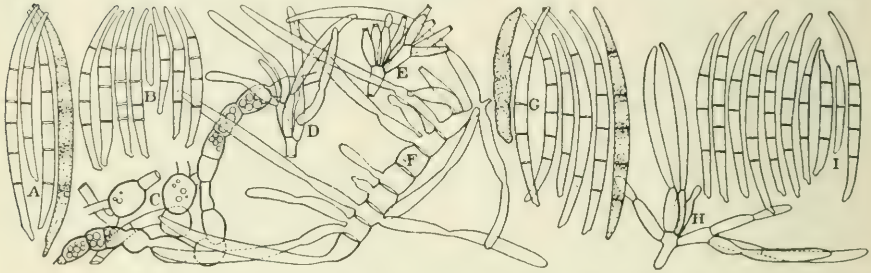

FIG. 11.—*Fusarium metacroum var. minus.* A, *Conidia from 18-days-old culture on hard lima-bean agar;* B, *conidia from the original specimen, potato stem, August 7, 1912;* C, *chlamydospore-like structures of hyphæ from 26- and 176-days-old cultures on corn agar;* D, *conidiophore from the original specimen, potato stem, August 7, 1912;* E, *conidiophores from 31-days-old culture on red raspberry cane plug;* F, *conidia producing numerous conidiophores from 10-days-old culture on hard lima-bean agar;* G, *normal conidia from 10-days-old culture on hard lima-bean agar;* H, *conidiophores from 31-days-old culture on red raspberry cane plug;* I, *conidia from 31-days-old culture on red raspberry cane plug. All conidia are from pseudopionnotes*

On hard lima-bean agar with 2 per cent glucose, culture eight days old:

Conidia: 3–septate, 17 per cent
4–septate, 17 per cent
5–septate, 66 per cent, 60 x 3.6 (53–65 x 3–4.2)μ

On potato tuber agar, culture ninety-eight days old:

Conidia: 0–septate, 5 per cent
1–septate, 5 per cent
2–septate, rare
3–septate, 20 per cent, 34 x 3.5 (26–39 x 3–4)μ
4–septate, 30 per cent, 42 x 3.8 (36–48 x 3.5–4.1)μ
5–septate, 40 per cent, 46 x 3.9 (40–54 x 3.5–4.1)μ

On rye grain, culture sixty-five days old:
Conidia: 3–septate, 20 per cent, 41 x 3.2 (36–46 x 2.9–4)μ
4–septate, 17 per cent, 48 x 3.3 (40–54 x 2.9–4)μ
5–septate, 63 per cent, 52 x 3.7 (40–60 x 3–4.1)μ

On hard lima-bean agar, culture ten days old:
Conidia: 1–septate, very few
3–septate, 11 per cent, 50 x 3.3 (29–61 x 3–3.7)μ
4–septate, 4 per cent
5–septate, 85 per cent, 60 x 3.5 (45–67 x 3.1–4)μ

Average of the above measurements:
Conidia: 0– to 2–septate, 2 per cent, 20 x 2.5μ
3–septate, 23 per cent, 42 x 3.2μ
4–septate, 20 per cent
5–septate, 55 per cent, 54 x 3.6μ
6–septate, very rare, 61 x 3.4μ

14. *Fusarium subulatum* Ap. et Wr. (Fig. 1w; Fig. 12, G to J; Pl. II, fig. 11; Pl. VII, fig. 4)

Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. 8:118–132, Pl. II, figs. 65 to 87. 1910. Wollenweber, H. W., Phytopath. 3:32, fig. 1N. 1913.

Conidia slightly elliptically curved, typically of nearly even diameter for the greater part of their length, very gradually attenuate toward both ends, slightly pedicellate, typically 5–septate, 58 x 3.64 (48–65 x 3.4–3.85)μ, usually in numerous, sometimes converging sporodochia ($\frac{1}{4}$ to 1 millimeter in diameter), produced near substratum and lower aërial mycelium, from pink-flesh to apricot-buff and from coral red to brick red in color, with darker shades in old moist cultures; chlamydospores absent; aërial mycelium typically present, at first hyaline, then testaceous color and other hues of red; on various agars from vinaceous tawny and madder brown to pomegranate purple and Eugenia red.

Hab. A cosmopolitan saprophyte, of very wide occurrence on dead substrata, in soil and water, also parasitic on cereals and on tubers of *Solanum tuberosum*.

The organism was twice isolated by the writer from rotted potato tubers, and the strains were compared with a culture of the originally described organism obtained through the courtesy of Dr. Wollenweber and proved to be identical. The writer's measurements of conidia of the original strain are as follows:

On red raspberry cane plug, culture twenty-seven days old:
Conidia: 3–septate, 10 per cent, 55 x 2.9μ
4–septate, 20 per cent, 60.4 x 3 (54–76 x 2.6–3.5)μ
5–septate, 70 per cent, 65 x 3.4 (57–76 x 2.6–4.1)μ

On potato tuber plug, culture ninety-nine days old:
Conidia: 0– to 2–septate, none to very few
3–septate, 35 per cent, 33 x 3.7 (27–40 x 3–4)μ
4–septate, 20 per cent, 38 x 3.7 (32–42 x 3–4.1)μ
5–septate, 45 per cent, 48 x 3.85 (42–58 x 3.5–4.1)μ
6–septate, rare, 57 x 4μ (only one measured)

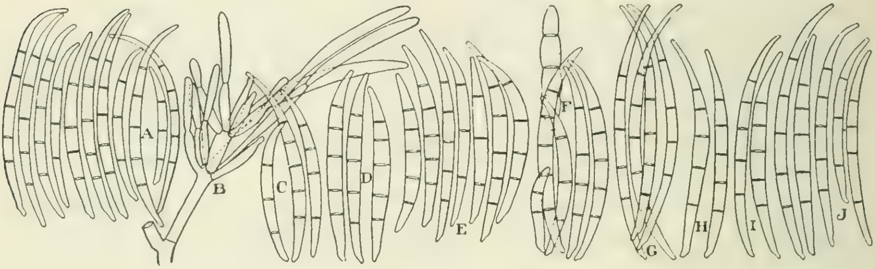

FIG. 12.— A-F, *Fusarium subulatum var. brevius:* A, *Conidia,* B, *conidiophore, from small sporodochium of 31-days-old culture on red raspberry cane plug;* C, *sporodochial conidia from 113-days-old culture on potato stem plug;* D, *pseudopionnotal conidia from 38-days-old culture on whole steamed potato tuber;* E, *sporodochial conidia from 65-days-old culture on rye straw;* F, *aërial conidia from 10-days-old culture on hard lima-bean agar.*

G-J, *F. subulatum:* G, *Sporodochial conidia from 31-days-old culture on red raspberry cane plug;* H, *semi-dry conidia from sporodochium of 38-days-old culture on steamed potato tuber;* I, *sporodochial conidia from 113-days-old culture on potato stem plug;* J, *sporodochial conidia from 65-days-old culture on rye grain*

On potato stem plug, culture one hundred and thirteen days old:
Conidia: 3–septate, 5 per cent, about 35 x 3μ (only a few measured)
4–septate, 4 per cent
5–septate, 91 per cent, 54 x 3.6 (47–65 x 3.2–3.9)μ

On steamed potato tuber, culture thirty-eight days old:
Conidia: 3–septate, 3 per cent, about 40 x 3.5μ
4–septate, 8 per cent, about 48 x 3.6μ
5–septate, 89 per cent, 55 x 3.7 (37–63 x 3.5–3.9)μ
6–septate, few, same measurement as 5–septate

On rye grain, culture sixty-five days old:

Conidia: 3–septate, 4 per cent, 52 x 3.4 (42–55 x 2.9–4)μ
4–septate, 15 per cent, 54 x 3.5 (42–67 x 2.9–4.1)μ
5–septate, 81 per cent, 64 x 3.7 (45–75 x 2.9–4.2)μ

On hard lima-bean agar, culture ten days old:

Conidia: 0–septate, rare
2–septate, very rare
3–septate, 11 per cent, 45 x 3.1 (33–60 x 3–3.5)μ
4–septate, 19 per cent
5–septate, 70 per cent, 60 x 3.6 (53–67 x 3.1–4)μ
9–septate, rare, 69 x 5.7μ (only one measured)

Average of the above measurements:

Conidia: 0–septate, none to rare
1– and 2–septate, none to rare
3–septate, 11 per cent, 43.3 x 3.3μ
4–septate, 15 per cent
5–septate, 74 per cent, 58 x 3.64μ
6– to 9–septate, none to rare

The averages of Appel and Wollenweber's measurements for the same organism are as follows:

Conidia: 1–septate, rare

$$\left.\begin{array}{l}\text{3–septate}\\ \text{4–septate}\end{array}\right\}\text{28 per cent}\left\{\begin{array}{l}36.5\text{ x }3.86\mu\\ 51.5\text{ x }3.5\mu\end{array}\right.$$

5–septate, 62 per cent, 61 x 3.86μ
6–septate, 9 per cent, 69 x 4μ
7–septate, 1 per cent

This shows the 5–septate conidia to be in about the same proportion and of about the same size as found by the writer.

15. *Fusarium subulatum* Ap. et Wr. var. **brevius** n. var. (Fig. 12, A to F; Pl. II, fig. 12; Pl. VII, fig. 3)

Conidia and fruiting forms of the same type as those of *F. subulatum;* chlamydospores also absent; 5–septate conidia average 50 x 3.8 (41–58 x 3.1–4.2)μ in size.

Hab. On rotted tuber of *Solanum tuberosum*, Ithaca, New York.

Differs from *F. subulatum* Ap. et Wr. mainly in shorter conidia, absence of carmine color in substratum, and high, better-developed, aërial mycelium.[39]

The measurements of the conidia on various media are as follows:

On red raspberry cane plug, culture thirty-one days old:

Conidia: 3–septate, 35 per cent, 40 x 2.7 (29–49 x 2.3–3.3)μ
4–septate, 20 per cent, 51 x 3 (45–53 x 2.3–3.4)μ
5–septate, 45 per cent, 55 x 3.1 (48–60 x 2.9–3.8)μ

On potato tuber plug, culture ninety-nine days old (in general, spores more or less deteriorated):

Conidia: 1–septate, 1 per cent
3–septate, 33 per cent, 30 x 3.6 (21–42 x 3–4)μ
4–septate, 20 per cent, 36 x 3.9 (33–42 x 3.5–4.1)μ
5–septate, 46 per cent, 41 x 4 (35–48 x 3.5–4.1)μ
6–septate, rare, 43 x 4.1μ (only one measured)

On potato stem plug, culture one hundred and thirteen days old:

Conidia: 3–septate, 67 per cent, 34 x 3 (28–49 x 2.9–3.6)μ
4–septate, 20 per cent, 42 x 3.3 (37–48 x 3–3.8)μ
5–septate, 13 per cent, 44.6 x 3.4 (38–47 x 3–3.8)μ

On whole steamed potato tuber, culture thirty-eight days old:

Conidia: 3–septate, 25 per cent, 37 x 3.8 (31–42 x 3.5–4)μ
4–septate, 35 per cent, 43 x 4 (36–49 x 3.5–9.2)μ
5–septate, 40 per cent, 47 x 4.1 (40–56 x 3.5–4.4)μ
6–septate, few, 52 x 4.4 (only a few measured)

On rye grain, culture sixty-five days old:

Conidia: 3–septate, 16 per cent, 42 x 4 (36–49 x 3.5–4.3)μ
4–septate, 17 per cent, 45 x 4.1 (36–54 x 3.5–4.3)μ
5–septate, 67 per cent, 51 x 4.2 (43–58 x 3.7–4.7)μ

On hard lima-bean agar, culture ten days old:

Conidia: 0–septate, very rare
1–septate, very rare
2–septate, very rare
3–septate, 24 per cent, 41 x 3.3 (36–48 x 3–3.5)μ
4–septate, 20 per cent
5–septate, 56 per cent, 52 x 3.9 (42–59 x 3.2–4.1)μ

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[39] This grows straight up and out from the point of inoculation in a plate culture on potato agar in the first week of its growth, while the aërial mycelium in *F. subulatum* is always of a more or less loose, feltlike character, uniformly medium short over the surface of the colony.

On medium potato agar, culture ten days old:
Conidia: 3–septate, 9 per cent, about 45 x 3.3 (only two measured)
4–septate, 3 per cent
5–septate, 88 per cent, 58 x 3.5 (47–64 x 3.1–4)μ

Average of the above measurements:
Conidia: 0– to 2–septate, absent or more or less rare
3–septate, 30 per cent, 38.4 x 3.4μ
4–septate, 19 per cent
5–septate, 51 per cent, 49.8 x 3.76μ
6–septate, absent to 1 per cent, 47.5 x 4.25μ

16. **Fusarium effusum** n. sp. (Fig. 13; Pl. vii, fig. 6)

Conidia gradually pointed toward apex, distinctly but not prominently pedicellate, typically 5–septate, 50 x 4.3 (44.5–57 x 3.9–4.5)μ, often 3– to 7–, seldom 1– to 2–septate; single or in indistinct pseudopionnotes and in large (on oats, wheat, and the like, about ½ centimeter in diameter) plectenchymic sporodochia; shape of conidia of nearly the same type, in all stages and in mass, of salmon color and its tints; aërial mycelium typically well developed, though on an agar may be resupinate, uniform, without any differentiated tufts or strands, from white to cream and tints of salmon color; substratum, on glucose agar, from chamois to morocco red, and on glucose-free agar, from colorless to Eugenia red; typical spore germination by straight, unbranched tubes (Fig. 13 j); mycelium in young colony (Fig. 13 h) typically composed of nearly straight, sparse in number, and more or less regular, branches.

Hab. On dry tubers of *Solanum tuberosum*, Minnesota.

Latin description.—Conidiis gradatim in apicem acutis, distincte sed non insignite pedicellatis, typice 5–septatis, 50 x 4.3 (44.5–57 x 3.9–4.5)μ, saepe 3–7–, raro 1–2–septatis; continuis vel in indistinctis pseudopionnotibus atque in magnis (in avena, tritico, etc., circa ½ cm. diam.) plectenchymicis sporodochiis; conidiis prope eodem in omnibus gradibus typo, in totum "salmon-color" (R); aerio mycelio typice plene maturo, sed in agare interdum resupinato, uniformi, sine ullis discretis cristis aut fibris, ex albo "cream color" (R) "salmon color" (R) vel simili colore; substrato in agare glucoso e "chamois" (R) "morocco red" (R), in agare non glucoso ex hyalino "Eugenia red" (R); sporis per rectos et non ramosos cylindros typice germinatis (Fig. 13 j); mycelio in colonia juveni ex prope rectis, sparsis, plus minusve regularibus ramis composito.

Hab. In tuberibus aridis Solani tuberosi, Minnesota, Amer. bor.

The fungus was isolated in association with *F. Solani* from an old rotted potato tuber from Minnesota. Measurements of conidia from cultures on different media are as follows:

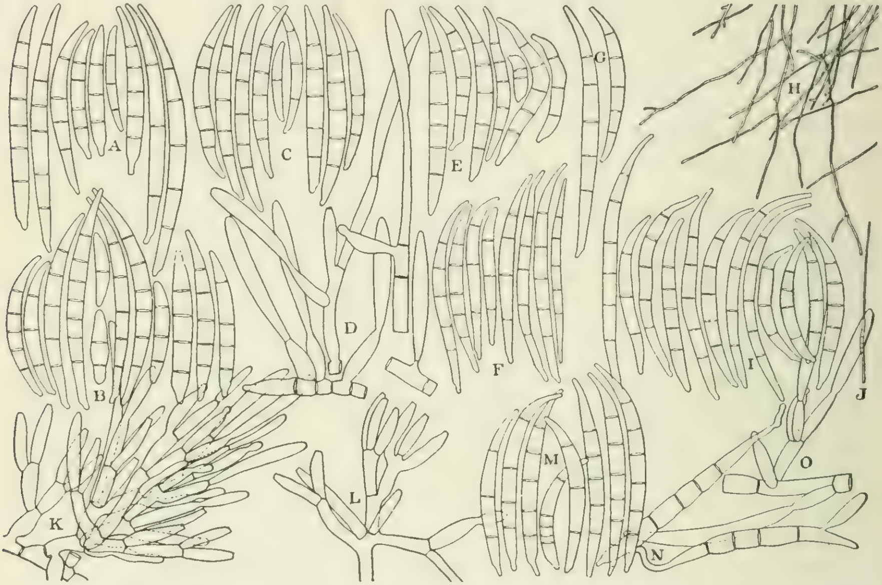

FIG. 13.—*Fusarium effusum.* A, *Pseudopionnotal conidia from 7-days-old culture on hard lima-bean agar with 2 per cent glucose;* B, *pseudopionnotal conidia from 13-days-old culture on hard lima-bean agar (rare forms of conidia shown at the center);* C, *pseudopionnotal conidia,* D, *conidiophores, from 9-days-old culture on medium potato agar;* E, *sporodochial conidia from 24-days-old culture on potato tuber plug;* F, *sporodochial conidia from 76-days-old culture on red raspberry cane plug;* G, *typical sporodochial conidia from 107-days-old culture on potato stem plug;* H, *character of colony growth (magnification 80 times),* I, *sporodochial conidia, from 34-days-old culture on rye grain;* J, *character of spore germination from potato-decoction hanging drop in van Tieghem cell (magnification 80 times);* K, L, *conidiophores,* M, *normal conidia,* N, *anastomosed conidia,* O, *conidiophores, from 36-days-old culture on corn meal*

On slightly acidified hard potato agar, culture ten days old; conidia from pseudopionnotes:

Conidia: 2–septate, rare
3–septate, 50 per cent, 34 x 4.1 (28–47 x 3–4.7)μ
4–septate, 10 per cent, 45 x 4.2μ
5–septate, 40 per cent, 50 x 4.5 (42–65 x 3.5–5.3)μ

On red raspberry cane plug, culture seventy-six days old; conidia from a sporodochium:

Conidia: 3–septate, 35 per cent, 42 x 3.3 (32–48 x 2.7–4)μ
4–septate, 15 per cent, 47 x 3.6 (42–53 x 3.2–4.3)μ
5–septate, 50 per cent, 49 x 3.9 (38–58 x 3.2–4.4)μ
6–septate, very rare

On hard lima-bean agar, 2 per cent glucose, culture seven days old; conidia from mycelium:

Conidia: 1–septate, 1 per cent
2–septate, 5 per cent
3–septate, 19 per cent, 39 x 4.2 (28–50 x 3.9–4.8)μ
4–septate, 10 per cent
5–septate, 60 per cent, 57 x 4.3 (52–72 x 4–5.3)μ
6–septate, 5 per cent, 62 x 4.7 (49–72 x 4.3–5.3)μ
7–septate, rare, 72 x 4.3μ (only one measured)
8–septate, exceptional, 87.5 x 5.8μ (only one measured)

On potato tuber plug, culture twenty-four days old; conidia from a sporodochium:

Conidia: 0–septate
1–septate, 2 per cent
2–septate, 1 per cent
3–septate, 12 per cent, 32 x 4.3 (28–36 x 3.9–4.4)μ
4–septate, 21 per cent, 34 x 4.5 (29–41 x 4.1–4.7)μ
5–septate, 61 per cent, 44.5 x 4.5 (35–51 x 4.1–4.8)μ
6– and 7–septate, 3 per cent, 53 x 4.7 (49–54 x 4.3–5.2)μ

On potato stem plug, culture one hundred and seven days old; conidia from a sporodochial mass (spores mostly with deteriorated ends):

Conidia: 3–septate, 5 per cent
4–septate, 15 per cent
5–septate, 80 per cent, 49 x 4 (35–63 x 3.5–4.7)μ

On hard lima-bean agar, culture ten days old; conidia from aërial mycelium:

Conidia: 1–septate, rare, 21 x 3.1μ (only one measured)
3–septate, 11 per cent, 36.2 x 4 (28–42 x 3.1–4.8)μ

4–septate, 4 per cent
5–septate, 81 per cent, 50.5 x 4.5 (38–60 x 3.9–4.8)μ
6–septate, 4 per cent, 56 x 4.7 (48–64 x 4.4–5.2)μ
7–septate, rare, 62 x 5.2μ (only one measured)

On the same medium as above, culture sixteen days old; conidia from aërial mycelium and indistinct pseudopionnotes:

Conidia: 1–septate, 1 per cent, 18 x 3.5μ (only a few measured)
2–septate, 1 per cent, 24 x 4μ (only a few measured)
3–septate, 12 per cent, 33 x 4 (21–44 x 2.9–4.8)μ
4–septate, 6 per cent
5–septate, 71 per cent, 50 x 4.3 (38–62 x 3.5–4.7)μ
6–septate, 7 per cent, 60 x 4.7 (55–64 x 4.5–4.8)μ
7–septate, 2 per cent, 62 x 4.7 (59–65 x 4.4–4.8)μ

Average of the above measurements:

Conidia: 1–septate, less than 1 per cent, 18 x 3.5μ
2–septate, 2 per cent, 24 x 4.0μ
3–septate, 20 per cent, 36 x 4.0μ
4–septate, 12 per cent
5–septate, 63 per cent, 50 x 4.3μ
6– and 7–septate, 3 per cent, 68 x 4.8μ

F. effusum, especially in its sporodochial stage, much resembles the following other species: *F. subulatum*, *F. lucidum*, *F. biforme*, *F. diversisporum*. From *F. subulatum* it can be at once distinguished by the diameter of the conidia and by the larger size of sporodochia.[40] From *F. lucidum* it differs primarily by the common presence of 6– and 7–septate conidia, which are absent in *F. lucidum*, and by denser red substratum than that of the latter species. From *F. biforme* it differs mainly by absence of the long, 9– or more septate, conidia in pseudopionnotes, and also by absence of arthrosporial conidial form, the pluriseptate conidia and arthrosporial conidia on aërial mycelium being more or less common in *F. biforme*. From *F. diversisporum* it differs by absence of arthrosporial conidia, which are typically produced on aërial mycelium of *F. diversisporum*.

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[40] Sporodochia of *F. subulatum* as a rule are small, but on whole steamed potato tubers they may be as large as those of *F. effusum*.

17. **Fusarium truncatum** n. sp. (Figs. 1, C_1 to E_1, and 14; Pl. VII, fig. 1)

Conidia typically sickle-shaped, gradually pointed toward the apex, slightly broader at or just above the middle, distinctly pedicellate, 3– to 5–septate; 3–septate averaging 35 x 3.7 (31.5–42 x 3.4–3.9)μ, 5–septate averaging 45 x 3.9 (43–48 x 3.4–4.2)μ; from cinnamon and terra cotta to carmine-pomegranate purple in color; conidiophores from loose to dense, bushlike, single or in from small to large (up to $\frac{1}{2}$ centimeter in diameter) sporodochia; aërial mycelium always well developed, mostly composed of fine but macroscopically distinct threads, from white to slightly carmine near substratum; color of substratum, on hard potato agar rich in glucose, varies from pale cinnamon and diffuse salmon hues to corinthian and brick red, more or less distinctly zonate.

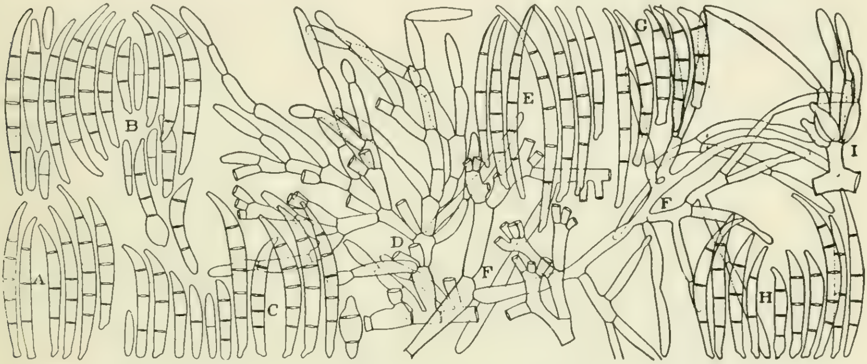

FIG. 14.—*Fusarium truncatum.* A, *Sporodochial conidia from 60-days-old culture on red raspberry cane plug;* B, *aërial conidia from 13-days-old culture on hard potato agar;* C, *aërial conidia (some with distinctly truncate basal cell) from 14-days-old culture on medium potato agar;* D, *conidiophore from 13-days-old culture on hard potato agar;* E, *sporodochial conidia from 14-days-old culture on medium potato agar;* F, *conidiophores from 47-days-old culture on potato tuber plug;* G, *sporodochial conidia from 37-days-old culture on hard oat agar;* H, *conidia from aërial mycelium from 47-days-old culture on potato tuber plug;* I, *conidiophore from 4-days-old colony in petri dish on hard potato agar with 10 per cent glucose*

Hab. On rotted tubers of *Solanum tuberosum*, New York State.

Latin description.—Conidiis typice falciformibus, gradatim in apicem acutis, paulo latioribus medio vel subinde supra medium, distincte pedicellatis 3–5–septatis; 3–septatis plerumque 35 x 3.7 (31.5–42 x 3.4–3.9)μ, 5–septatis plerumque 45 x 3.9 (43–48 x 3.4–4.2)μ; e "cinnamon" (R) et "terra cotta" (R) "carmine" (R) et "pomegranate purple" (R); conidiophoris laxis demum dense, fruticosis, continuis vel in parvis magnisve (usque ad ½ cm. diam.) sporodochiis; aerio mycelio semper plene maturo, ex hyphis subtilibus sed macroscopice distinctis composito, ex albo paulum "carmine" (R) prope substratum; substrato in duro agare glucoso Solani tuberosi e pallide "cinnamon" (R) et "salmon color" (R) diffuse "corinthian" et "brick red" (R), plus minusve distincte zonato.

Hab. In tuberibus putridis Solani tuberosi, New York, Amer. bor.

Measurements of conidia from cultures on different media are as follows:

On slightly acidified hard potato agar, culture thirteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 7 per cent
1–septate, 54 per cent
2–septate, 6 per cent
3–septate, 22 per cent, 31.5 x 3.75 (21–45 x 3.1–4.1)μ
4–septate, 4 per cent, 40 x 4.1 (32–49 x 3.5–4.7)μ
5–septate, 7 per cent, 43 x 4.1 (33–53 x 3.9–4.7)μ
6–septate, rare, 45 x 4.5μ (only two measured)

On potato tuber plugs, culture forty-six days old; conidia from a sporodochium 2 millimeters in diameter:

Conidia: 3–septate, 63 per cent, 37 x 3.4 (27–49 x 3–3.6)μ
4–septate, 22 per cent, 41 x 3.7 (40–45 x 3.5–4)μ
5–septate, 15 per cent, 43 x 3.7 (40–45 x 3.5–4.1)μ

On red raspberry cane plug, culture sixty days old; conidia from a sporodochium about 2 millimeters in diameter:

Conidia: 3–septate, 29 per cent, 37 x 3.9 (28–41 x 3.5–4.2)μ
4–septate, 31 per cent, 40 x 3.9 (35–43 x 3.5–4.2)μ
5–septate, 40 per cent, 44 x 4 (36–46 x 3.5–4.5)μ

On potato stem plug, culture eighty-three days old; conidia from a sporodochium about 1½ millimeters in diameter (many of the conidia much deteriorated):

Conidia: 3–septate, 20 per cent
4–septate, 16 per cent
5–septate, 64 per cent, 48 x 3.4 (44–51 x 3.1–3.7)μ

On soft potato agar, plate culture fourteen days old; conidia from thick pseudopionnotal mass near the inoculation point:

Conidia: 3–septate, 20 per cent, 42 x 3.4 (35–51 x 3–3.9)μ
4–septate, 13 per cent
5–septate, 67 per cent, 50 x 3.6 (43–65 x 3.1–3.9)μ

On same medium as above, plate culture also; conidia from a small sporodochium on aërial mycelium:

Conidia: 0–septate, rare
1–septate, 3 per cent, 16 x 3.1μ (only one measured)
2–septate, 2 per cent, 21 x 3.5μ (only one measured)
3–septate, 29 per cent, 33 x 3.8 (22–41 x 3.3–4.2)μ
4–septate, 19 per cent
5–septate, 47 per cent, 45 x 4.2 (36–64 x 4–4.4)μ

Average of the above measurements:

Conidia: 0–septate, about 1 per cent
1–septate, about 9 per cent
2–septate, about 1 per cent
3–septate, about 31 per cent, 34.8 x 3.7μ
4–septate, about 18 per cent
5–septate, about 40 per cent, 45 x 3.9μ
6–septate, very exceptional, 45 x 4.5μ (only two measured)

Conidia of *F. truncatum* often have a peculiar flat base, and this species can be separated from all the other Fusaria of potatoes by its typically (though not always) pomegranate purple to carmine conidial masses.

18. **Fusarium lucidum** n. sp. (Figs. 1v and 15; Pl. II, figs. 9 and 10; Pl. IV, fig. 12; Pl. VI, fig. 12)

Conidia typically ellipsoid, very gradually attenuate toward both ends, distinctly but not prominently pedicellate, 5–septate, 54 x 4.05 (43–63 x 3.7–4.7)μ, salmon, often of very bright hues, but paler or denser in from small to large (up to ½ centimeter in diameter) sporodochia; no chlamydospores; conidiophores typically more or less compound, bushlike; mycelium from white to pale cinnamon and pomegranate near substratum; when first isolated the fungus has a substratum, on potato agar without glucose from pale pink to tints of pomegranate, and on the same medium with glucose from clay to buckthorn brown.

Hab. On rotted tubers of *Solanum tuberosum*, New York State.

Differs from *F. effusum* mainly by absence or very rare occurrence of 6– and 7–septate conidia.

Latin description.—Conidiis typice ellipsoidalibus, maxime in utrosque terminos gradatim attenuatis, distincte sed non insignite pedicellatis, 5–septatis, 54 x 4.05 (43–63 x 3.7–4.7)μ, "salmon-color" (R) magnis, in parvis (usque ad $\frac{1}{2}$ cm. diam.) sporodochiis; nullis chlamydosporis; conidiophoris typice plus minusve compositis, fruticosis; mycelio ex albo "pale cinnamon" (R) vel "pomegranate" (R) prope substratum; primum substrato, in agare Solani tuberosi non glucoso, e pallide rubello "pomegranate" (R) vel simili colore, in eodem agare glucoso, ex argillaceo "buckthorn-brown" (R).

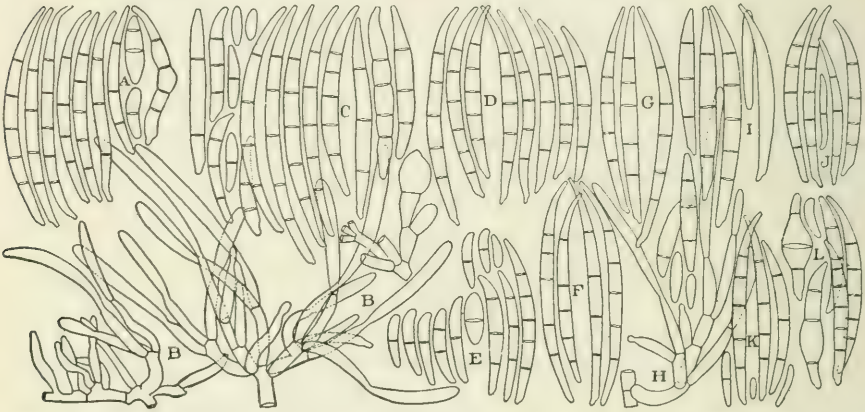

FIG. 15.—*Fusarium lucidum.* A, *Sporodochial conidia,* B, *conidiophores, from 47-days-old culture on wheat kernels;* C, *pseudopionnotal conidia from 11-days-old culture on slightly acidified hard potato agar;* D, *sporodochial,* E, *aërial, conidia from 75-days-old red raspberry cane plug;* F, *pseudopionnotal conidia from 36-days-old culture on hard oat agar;* G, *sporodochial conidia,* H, *conidiophore, from 20-days-old culture on hard lima-bean agar with 2 per cent glucose;* I, *pseudopionnotal conidia from 7-days-old culture on hard lima-bean agar with 2 per cent glucose;* J, *sporodochial conidia from 65-days-old culture on potato stem plug;* K, *sporodochial conidia from 24-days-old culture on potato tuber plug;* L, *sporodochial conidia from 82-days-old culture on potato tuber plug (the two at the left abnormal)*

Hab. In tuberibus putridis Solani tuberosi, New York, Amer. bor.

Measurements of conidia from cultures on different media are as follows:

On hard potato agar, slightly acidified, culture eleven days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare
1–septate, 11 per cent, 17 x 3.1 (12–22 x 2.9–3.5)μ
2–septate, 7 per cent, 28 x 3.8 (20–32 x 2.9–4.3)μ
3–septate, 68 per cent, 43.7 x 3.8 (33–67 x 3–4.4)μ
4–septate, 4 per cent, 55 x 4.2 (47–65 x 4–4.4)μ
5–septate, 10 per cent, 63 x 4.4 (57–72 x 4–4.8)μ

On red raspberry cane plug, culture seventy-five days old; conidia from aërial mycelium:

Conidia: 0–septate, rare
1–septate, 1 per cent
3–septate, 5 per cent, 43 x 3.8 (25–48 x 3.5–4.4)μ
4–septate, 14 per cent, 45 x 3.8 (38–54 x 3–4.6)μ
5–septate, 80 per cent, 51 x 4.2 (42–60 x 3.5–4.7)μ
6–septate, rare

On potato tuber plugs, culture ninety-two days old; conidia from a mass of minute sporodochia:

Conidia: 0–septate, rare
1–septate, 3 per cent
2–septate, 2 per cent
3–septate, 25 per cent, 31 x 3.8 (24–36 x 3–4.1)μ
4–septate, 17 per cent
5–septate, 53 per cent, 46 x 3.9 (36–58 x 3.5–4.7)μ
6–septate, rare, 55–60 x 3.8–4.7μ (only a few measured)

On hard lima-bean agar with 2 per cent glucose, culture seven days old; conidia from aërial mycelium:

Conidia: 0–septate, 40 per cent
1–septate, 35 per cent
2–septate, 5 per cent
3–septate, 14 per cent, 41 x 4.2 (33–44 x 3.5–4.8)μ
4–septate, 5 per cent, 48 x 4.6 (42–53 x 4–5.3)μ
5–septate, 1 per cent, 56 x 4.7 (53–58 x 4.3–4.9)μ (only five spores measured, the largest 60 x 5.7μ)

On the same medium as above, culture twenty days old; conidia from a small sporodochium:

Conidia: 3–septate, 1 per cent
4–septate, 12 per cent, 44 x 4 (40–57 x 3.5–4.4)μ
5–septate, 87 per cent, 54 x 3.9 (40–68 x 3.5–4.2)μ

On potato tuber plug, culture twenty-four days old; conidia from aërial mycelium:

Conidia: 3–septate, 10 per cent, 46 x 3.4μ (only a few measured)
4–septate, 30 per cent, 47 x 3.5 (44–50 x 3–3.8)μ
5–septate, 60 per cent, 51 x 3.7 (48–57 x 3.4–4.2)μ
6–septate, rare, 60 x 4.2μ (only one measured)

On potato stem plug, culture one hundred and seven days old; conidia from a sporodochial mass (much deterioated):

Conidia: 3–septate, 15 per cent
4–septate, 5 per cent
5–septate, 80 per cent, 56 x 3.8 (50–63 x 3.5–4.2)μ

On steamed whole potato tuber, culture forty-nine days old; conidia from a white, semi-dry, sporodochial mass:

Conidia: 3–septate, 50 per cent, 36 x 3.9 (24–43 x 3.5–4.1)μ
4–septate, 35 per cent
5–septate, 15 per cent, 43 x 4.1 (39–46 x 3.9–4.4)μ

On same medium as above; conidia from a red (old?) sporodochial mass, nearly converging into semi-pionnotal layer:

Conidia: 0–septate, very rare
3–septate, 8 per cent, 46 x 3.7 (24–50 x 3–4)μ
4–septate, 12 per cent, 48 x 3.8 (41–52 x 3.5–4.1)μ
5–septate, 80 per cent, 54 x 4 (43–62 x 3.5–4.3)μ
6–septate, very rare, about 57 x 4.2μ (only a few measured)

On medium soft potato agar, culture fourteen days old; conidia from pseudopionnotes:

Conidia: 3–septate, 28 per cent, 42 x 3.6μ (only four measured)
4–septate, 10 per cent
5–septate, 62 per cent, 58 x 4 (43–65 x 3.5–4.7)μ

On hard lima-bean agar, culture fourteen days old; conidia from a sheet of small sporodochial masses spread over the substratum:

Conidia: 3–septate, 3 per cent, 41 x 3.2μ (only three measured)
4–septate, 1 per cent
5–septate, 96 per cent, 60 x 3.8 (52–70 x 3.5–5)μ

On wheat grain culture fourteen days old; conidia from small, semi-dry sporodochia:

Conidia: 1–septate, rare
3–septate, 14 per cent
4–septate, 26 per cent
5–septate, 60 per cent, 56 x 3.7 (49–64 x 3.3–4.1)μ

Average of the above measurements:

Conidia: 0–septate, about 3 per cent
1–septate, about 4 per cent, 17 x 3.1μ
2–septate, about 1 per cent
3–septate, about 20 per cent, 41 x 3.7μ
4–septate, about 14 per cent
5–septate, about 58 per cent, 54 x 4.05μ
6–septate, very rare, 58 x 4.2μ

VI. SECTION **ARTHROSPORIELLA** n. sec.

Microconidia short and broad, spindle-shaped, 0– to 3–septate[41]; sporodochial macroconidia when present sickle-shaped, mostly 5–septate, of Roseum type; pseudopionnótal microconidia mostly 5– and 5– to 7–, often to 9– and more, septate, from slightly curved to straight and anguiform; true chlamydospores absent; aërial mycelium from white to pale buff and different hues of red and pink; color of substratum from clay to different hues of red.

The section is a connecting link between sections Roseum and Sporotrichiella (through *F. arthrosporioides*).

19. **Fusarium diversisporum** n. sp. (Fig. 16; Pl. VII, fig. 12)

Conidia varying from arthrosporial (short, spindle-shaped, and having an average measurement when 3–septate and on aërial mycelium of 28 x

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[41] These conidia, though often septate, represent an abbreviated type and thus can be termed microconidia. These microconidia are often referred to as arthrosporial because of their resemblance to the conidia of the genus Arthrosporium.

4.3μ) to sickle-shaped, 5-septate type dominant, in sporodochia and pseudopionnotes, measuring 48.5 x 3.63 (41–61 x 2.9–4.4)μ; in pseudopionnotes, 6- to 9-septate conidia are common, 60–100 x 4.7–5.2μ, from slightly curved to straight and anguiform, apically pointed, distinctly but not prominently pedicellate, in mass typically of light pink-cinnamon color; chlamydospores absent; sporodochia when present often of a large size (up to 3.7 centimeters in diameter); aërial mycelium typically well developed, of uniformly medium fineness, white; substratum, on potato agar rich in glucose, ranging from onion-skin pink and clay color when young to Saccardo's amber in old cultures.

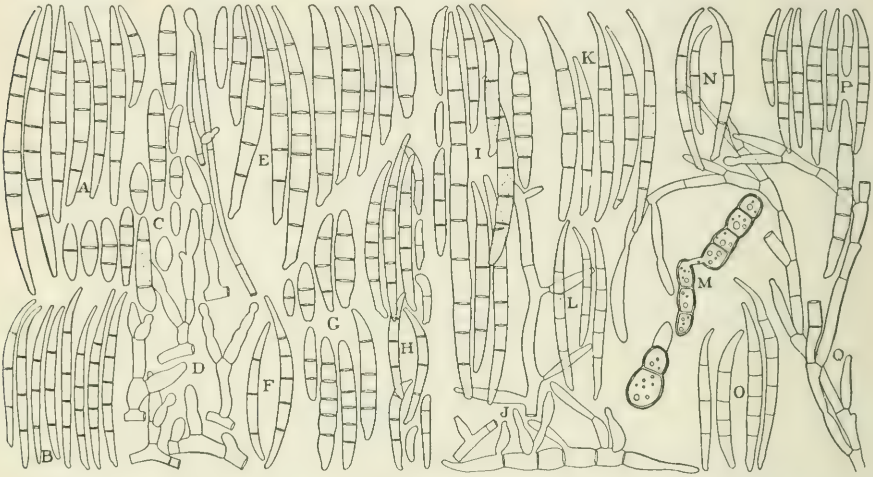

Fig. 16.—*Fusarium diversisporum.* A, *Pseudopionnotal conidia from 26-days-old culture on hard oat agar;* B, *sporodochial conidia,* C, *arthrosporial conidia,* D, *aërial conidiophores producing arthrosporial conidia, from 76-days-old culture on red raspberry cane plug;* E, *pseudopionnotal conidia from 8-days-old culture on hard lima-bean agar with 2 per cent glucose;* F, *typical sporodochial conidia from 113-days-old culture on potato stem plug;* G, *sickle-shaped, normal conidia,* H, *arthrosporial, more or less abnormal, conidia, from aërial mycelium of 45-days-old culture on whole steamed potato tuber;* I, *pseudopionnotal conidia,* J, *mycelial conidiophores and conidiophores produced directly on spores,* K, *conidia, from 10-days-old culture on medium potato agar;* L, *typical sporodochial conidia from 46-days-old culture on whole steamed potato tuber;* M, *chlamydospore-like structures in old conidia from 173-days-old culture on corn agar;* N, *sporodochial conidia from 42-days-old culture on rye straw;* O, *sporodochial conidia from 116-days-old culture on rye straw;* P, *sporodochial conidia from 24-days-old culture on potato tuber plug;* Q, *conidiophore from 24-days-old culture on potato tuber plug*

Hab. On rotted tubers of *Solanum tuberosum*, New York State, in close association with *F. metacroum.*

Latin description.—Conidiis ex arthrosporialibus (brevibus, falciformibus, atque, si 3-septata et in aerio mycelio, plerumque 28 x 4.3μ), falciformibus plerumque 5-septatis, 48.5 x 3.63 (41–61 x 2.9–4.4)μ in sporodochiis pseudopionnotibusque; in pseudopionnotibus, conidiis 6–9-septatis frequentibus, 60–100 x 4.7–5.2μ, parum curvatis rectis, demum anguiformibus, apice acutis, distincte sed non insignite pedicellatis, in totum typice pallide "pinkish cinnamon" (R); nullis chlamydosporis; sporodochiis, si exstant, saepe magnis (usque ad 3.7 cm. diam.); aerio mycelio typice plene maturo, uniformi mediocri subtilitate, albo; substrato—in Solani tuberosi agare perglucoso—in culturis juvenibus, "onion-skin pink" (R) vel "clay-color" (R), in culturis maturis "Saccardo's amber" (R).

Hab. In tuberibus putridis Solani tuberosi una cum F. metacroo, New York, Amer. bor.

Measurements of conidia from cultures on different media are as follows:

On slightly acidified hard potato agar, culture ten days old; conidia from thin pseudopionnotes:

Conidia: 0–septate, rare
1–septate, 7 per cent, 10 x 2.4μ
2–septate, 1 per cent, 20 x 2.9μ
3–septate, 36 per cent, 36 x 3.1 (29–46 x 2.9–3.5)μ
4–septate, 20 per cent, 49 x 3.15 (40–53 x 3–4)μ
5–septate, 35 per cent, 60 x 3.8 (50–70 x 3.5–4.2)μ
6– to 9–septate, 1 per cent (60–100 x 4–5μ)

On red raspberry cane plug, culture seventy-six days old; conidia from a small sporodochium:

Conidia: 3–septate, 57 per cent, 40 x 2.7(32–45 x 2.4–3)μ
4–septate, 28 per cent, 45 x 2.9(42–52 x 2.6–3.2)μ
5–septate, 15 per cent, 50 x 2.9(43–54 x 2.7–3.2)μ

On hard lima-bean agar, culture eight days old; conidia from a thin layer near substratum:

Conidia: 1–septate, 2 per cent
2–septate, 1 per cent
3–septate, 32 per cent, 37 x 3.9 (28–43 x 3.5–5.3)μ
4–septate, 9 per cent

5–septate, 55 per cent, 54 x 4.4 (40–61 x 3.5–5.5)μ

6–septate	1 per cent	61 x 4.9 (60–70 x 4.7–5.2)μ (only four measured)
7–septate		67 x 5 (60–79 x 4.7–5.2)μ (only four measured)
8–septate		76 x 4.8 (70–79 x 4.7–4.8)μ (only three measured)

On potato tuber plug, culture twenty-four days old; conidia from a sporodochium, many spores degenerated, only normal ones measured:

Conidia: 0–septate, none
1–septate, 4 per cent
2–septate, none
3–septate, 50 per cent, 32 x 3.1 (20–40 x 2.7–3.5)μ
4–septate, 22 per cent
5–septate, 24 per cent, 42 x 3.5 (35–51 x 3–3.6)μ

On potato stem plug, culture one hundred and thirteen days old; conidia from a sporodochium:

Conidia: 3–septate, 25 per cent, about 38 x 3.3μ
4–septate, 25 per cent, about 40 x 3.4μ
5–septate, 50 per cent, 42 x 3.5 (36–46 x 3–4.1)μ

On whole steamed potato tuber, culture forty-five days old:

(1) Conidia from aërial mycelium
Conidia: A — Arthrosporial type
1–septate, 10 per cent, about 10 x 3.9μ
2–septate, 3 per cent, 16 x 4μ
3–septate, 71 per cent, 28 x 4.3 (19–37 x 4–5)μ
4–septate, 6 per cent
5–septate, 10 per cent, 41 x 4.2 (37–46 x 4.1–4.5)μ
B — Sickle-shaped type
0–septate, 6 per cent, about 10 x 2.6μ
1–septate, 22 per cent, 16 x 2.8 (13–19 x 2.4–3)μ
2–septate, 4 per cent, about 21 x 2.9μ
3–septate, 40 per cent, 30 x 3.1 (20–34 x 2.9–3.5)μ
4–septate, 12 per cent, about 39 x 3.2μ
5–septate, 16 per cent, 45 x 3.3 (38–53 x 3–3.6)μ

(2) Conidia from small sporodochia converging into a nearly continuous layer

Conidia: 3–septate, 45 per cent, 33 x 3.1 (20–41 x 2.5–3.5)μ
4–septate, 28 per cent, 40 x 3.2μ (only a few measured)
5–septate, 27 per cent, 43 x 3.3 (36–50 x 2.9–4.1)μ

On hard lima-bean agar, culture thirteen days old; conidia from pseudopionnotal stage:

Conidia: 3–septate, about 12 per cent, 48 x 3μ (only three measured)
4–septate, about 8 per cent
5–septate, 80 per cent, 61 x 3.3 (50–70 x 3.1–3.5)μ

On hard oat agar, culture thirty-seven days old; conidia from aërial mycelium close to substratum:

Conidia: 1–septate, 1 per cent
3–septate, 20 per cent, 31 x 3.3μ
4–septate, 15 per cent
5–septate, 60 per cent, 45 x 3.7 (43–49 x 3.3–4)μ
6–septate, 4 per cent, 61 x 4.2μ (only one measured)

On same medium as above, culture twenty-four days old; conidia from pseudopionnotes:

Conidia: 3–septate, 10 per cent, 28 x 3.3μ
4–septate, 4 per cent
5–septate, 50 per cent, 51 x 4μ
6–septate, 30 per cent, 60.6 x 4.2μ
7– and 8–septate, 6 per cent, 75 x 43μ

Average of the above measurements:

Conidia: 0–septate, about 0.5 per cent, 10 x 3.25μ
1–septate, about 4 per cent, 12 x 3.0μ
2–septate, about 0.5 per cent
3–septate, about 35.5 per cent, 36 x 3.2μ
4–septate, about 15 per cent
5–septate, about 44 per cent, 48.5 x 3.63μ
6– to 9–septate, about 0.5 per cent, 60–100 x 4.7–5.2μ

The most characteristic features of the fungus are as follows: 0– to 3–septate, spindle-shaped conidia of aërial mycelium; 3– to 5–septate, very narrow sickle-shaped conidia of sporodochia, and comparatively broad and longer anguiform; 6- to 9-septate conidia of pseudopionnotal stage often occurring on various agars.

20. **Fusarium biforme** n. sp. (Figs. 1U and 17; Pl. VII, fig. 10)

Conidia of two forms: sporodochial conidia more or less uniformly ellipsoid, 3- to 5-septate, 3-septate measuring 39 x 3.3 (36-46 x 2.9-3.6)μ, 5-septate measuring 51 x 3.5 (43-60 x 3-4)μ; pseudopionnotal conidia 0- to 9- and even up to 12-septate, sometimes nearly straight or anguiform, 5-septate measuring 52 x 4.2μ, 6- to 9-septate measuring 45-84 x 4-5.8μ; conidia in mass typically salmon-colored; no chlamydospores; mycelium well developed, uniform, white in pseudopionnotal stage and from pink to pomegranate near substratum in sporodochial stage (on hard oat agar);[42] substratum from brick red to pomegranate (on oat and other agars).

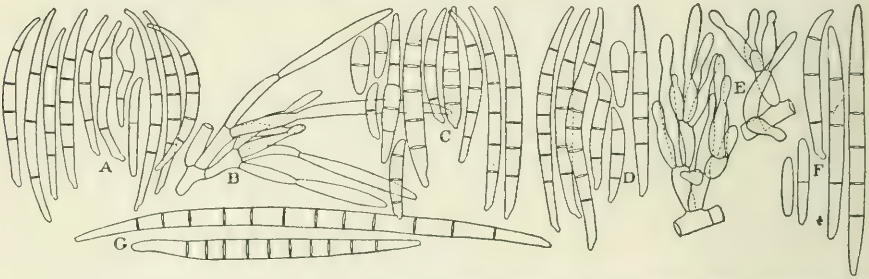

FIG. 17.—*Fusarium biforme.* A, *Conidia (sporodochium 3 millimeters in diameter),* B, *conidiophore, from 29-days-old culture on hard oat agar;* C, *conidia from aërial mycelium from 71-days-old culture on potato tuber plug;* D, *semi-pseudopionnotal conidia from 14-days-old culture on slightly acidified hard potato agar;* E, *conidiophores,* F, *typical conidia,* G, *largest and most highly septate conidia, from 10-days-old culture on rice*

Hab. On rotted tuber of *Solanum tuberosum*, together with *F. coeruleum*, Wisconsin.

Differs from *F. diversisporum* chiefly in color of mycelium and of substratum on hard oat agar and other agars, and in absence of a uniform and typical arthrosporial stage of aërial conidia, although single conidia of that type occur here also. Differs from *F. diffusum* by the presence of 8- to 12-septate conidia.

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[42] On the same medium *F. diversisporum* remains from white to clay in color.

Latin description.—Conidiis biformibus: conidiis sporodochialibus, plus minusve aequabiliter ellipsoidalibus, 3–5–septatis: 3–septatis, 39 x 3.3 (36–46 x 2.9–3.6)μ; 5–septatis 51 x 3.5 (43–60 x 3–4)μ; conidiis pseudopionnotalibus 0–9–septatis vel etiam 12–septatis, interdum prope rectis vel anguiformibus; 5–septatis, 52 x 4.2μ; 6–9–septatis, 45–84 x 4–5.8μ; conidiis in totum typice "salmon-color" (R); nullis chlamydosporis; mycelio plene maturo, uniformi, albo in pseudopionnotum gradu, ex rubello "pomegranate" (R) in gradu sporodochiali (in durae avenae agare); substrato e "brick red" (R) "pomegranate" (R) (in avenae agaribus et aliis agaribus).

Hab. In tuberibus putridis Solani tuberosi una cum F. coeruleo, Wisconsin, Amer. bor.

Measurements of the conidia on different media are as follows:

On corn agar, culture twenty-six days old; conidia from a thin layer directly on substratum;

Conidia: 1– to 4–septate, 20 per cent (about an equal number of each)
5–septate, 45 per cent, 60 x 3.6 (50–70 x 3.4–3.8)μ
6– to 9–septate, 35 per cent, 80 x 4.4 (77–88 x 4.1–4.7)μ

On hard potato agar, slightly acidified, culture fourteen days old; conidia from aërial mycelium close to substratum:

Conidia: 0–septate, 0.5 per cent, 12 x 3μ
1–septate, 3 per cent, 19 x 3μ
2–septate, rare, 25 x 3.2μ
3–septate, 5 per cent, 32 x 3.7 (24–51 x 3.5–4.2)μ
4–septate, 2.5 per cent
5–septate, 80 per cent, 51.5 x 4.3 (40–74 x 4–5.2)μ
6–septate, 6 per cent, 58 x 4.7 (50–75 x 4.3–5.3)μ
7– and 8–septate, 3 per cent, 68 x 4.7 (55–80 x 4.3–5.3)μ
9– to 11–septate, rare, 84 x 5.9μ (only a few measured)

On potato tuber plug, culture seventy-one days old; conidia from aërial mycelium close to substratum:

Conidia: 0–septate, 2 per cent, 9 x 3μ
1–septate, 6 per cent, 14 x 3.3μ, a few 15 x 5.7μ
2–septate, 3 per cent, 20 x 3.4μ
3–septate, 30 per cent, 27.5 x 3.9 (19–39 x 3.5–4.1)μ
4–septate, 12 per cent

5–septate, 44 per cent, 43 x 4.1 (31-54 x 3.9–5.2)μ
6–septate, 2 per cent, 49 x 4.3 (45–72 x 4–4.9)μ
7–septate, 1 per cent, 60 x 4.8 (59–73 x 4.1–5.3)μ
8– and 9–septate, rare, 60–78 x 4.5–5.8μ (only a few measured)

On hard lima-bean agar with 2 per cent glucose, culture seven days old; conidia from aërial mycelium:

Conidia: 0– and 1–septate, 79 per cent
3–septate, 15 per cent, 36 x 4.6 (33–41 x 4.3–4.7)μ
4–septate, 5 per cent
5–septate, 1 per cent, 54 x 4.7 (53–66 x 4.3–5.3)μ

On hard oat agar, culture twenty-eight days old; conidia from a large (3 millimeters in diameter) sporodochium:

Conidia: 1–septate, 3 per cent, about 28 x 3 (28–37 x 2.3–3)μ
2–septate, 1 per cent
3–septate, 45 per cent, 39 x 3.3 (36–46 x 2.9–3.6)μ
4–septate, 16 per cent
5–septate, 35 per cent, 51 x 3.5 (43–60 x 3–4)μ

Average of the above measurements for all non-sporodochial conidia:

Conidia: 0–septate, about 9 per cent, 11 x 3μ
1–septate, about 11 per cent, 16.5 x 3.15μ
2–septate, about 2 per cent
3–septate, about 20 per cent, 32 x 4.1μ
4–septate, about 8 per cent
5–septate, about 41 per cent, 52 x 4.2μ
6– to 9–septate, about 9 per cent, 45–84 x 4–5.8μ

The organism when first isolated had large sporodochia (from 2 to 5 millimeters in diameter), and the substratum and mycelium near it were from rose to pomegranate in color; later the color of mycelium and substratum faded and no sporodochia were produced at all; during the last year its original characters — both color and sporodochia — reappeared. Under just what influence loss of characters and their reappearance took place the writer is unable to say, although it seems that a slightly acidified and relatively dry medium actually helped to bring the fungus to the original conditions.

21. **Fusarium anguioides** n. sp. (Figs. 1T and 18; Pl. VI, fig. 11)

Conidia of diverse type, ranging from arthrosporial (short spindle-shaped, with more or less rounded ends, 0- to 3-septate) to typically slightly curved or nearly straight and anguiform, 1- to 15-septate; 1- and 3-septate conidia typical for the first form and measuring 27 x 4.4 (20–38 x 3.9–5.3)μ; for the other form the conidia commonly measuring as follows:

5-septate, 51 x 4.2 (47–68 x 3.9–4.6)μ
6- and 7-septate, 76 x 4.6 (65–86 x 4.2–5.2)μ
8- and 9-septate, 89 x 4.86 (80–102 x 4.3–5.8)μ

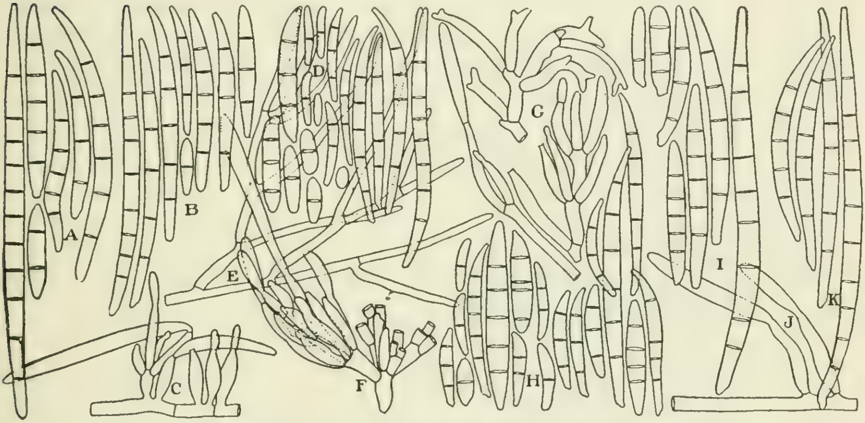

FIG. 18.—*Fusarium anguioides.* A, *Pseudopionnotal conidia from 6-days-old culture on slightly acidified hard potato agar;* B, *conidia from 15-days-old culture on wheat grain;* C, *conidiophores from 6-days-old culture on slightly acidified hard potato agar;* D, *conidia from 46-days-old culture on wheat grain;* E, *conidiophore from 15-days-old culture on wheat grain;* F *and* G, *conidiophores,* H, *conidia, from 62-days-old culture on red raspberry cane plug;* I, *pseudopionnotal conidia from 11-days-old culture on medium potato agar;* J, *conidiophores from 62-days-old culture on red raspberry cane plug;* K, *pseudopionnotal conidia from 13-days-old hard lima-bean agar*

Color of conidia in pseudopionnotal layer, on glucose potato agar, ranging from light pinkish cinnamon to cinnamon; arthrosporial conidia of common occurrence on aërial mycelium, but often the latter, especially on different agar, nearly absent, when a thin spore layer, pseudopionnotes, is produced for which anguiform conidia are typical.

Hab. On rotted tuber of *Solanum tuberosum* from Castile, New York, in association with *F. arcuosporum.*

In pseudopionnotal stage *F. anguioides* is much like *F. biforme,* but has no sporodochial stage, or rather no macroscopically observable sporodochia.

Latin description.—Conidiis variis typis, interdum arthrosporialibus (brevibus, fusiformibus, terminis plus minusve rotundatis, 0–3–septatis), interdum typice paulum curvatis vel prope rectis anguiformibusque, 1–15–septatis; conidiis primo typo typice 1–vel 3–septatis, 27 x 4.4 (20–38 x 3.9–5.3)μ; conidiis altero typo: 5–septatis plerumque 51 x 4.2 (47–68 x 3.9–4.6)μ; 6–7–septatis plerumque 76 x 4.6 (65–86 x 4.2–5.2)μ; 8–9–septatis plerumque 89 x 4.86 (80–102 x 4.3–5.8)μ. Conidiis in strato pseudopionnotali, in agari glucoso Solani tuberosi e pallide "pinkish-cinnamon" (R) "cinnamon" (R); saepe conidiis arthrosporialibus in aerio mycelio, sed hoc mycelio saepe — imprimis in alio agari — prope absente, quae cum ita sint tenues sporarum strati, pseudopionnotes, oriuntur cum conidiis typice anguiformibus.

Hab. In tuberibus putridis Solani tuberosi, una cum F. arcuosporo, Castile, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On red raspberry cane plug, culture sixty-two days old; conidia from aërial mycelium close to substratum:

Conidia: 0–septate, 3 per cent
1–septate, 19 per cent, 17 x 3 (12–22 x 2.5–4)μ
2–septate, 9 per cent
3–septate, 43 per cent, 30 x 3.5 (17–42 x 3–4.8)μ
4–septate, 7 per cent
5–septate, 18 per cent, 47 x 4.2 (35–55 x 3–5.2)μ, the thickest 50 x 5.9μ
6–septate, 1 per cent
7–septate, rare, the longest 75 x 4.8μ

On hard potato agar, culture eleven days old; conidia from thin pseudopionnotes:

Conidia: 1–septate, 3 per cent, 18 x 4.3μ (only a few measured)
2–septate, 2 per cent
3–septate, 20 per cent, 29 x 4.5 (20–35 x 4–6.5)μ, the broadest 34 x 6.5μ
4–septate, 10 per cent

5–septate, 44 per cent, 52 x 4.6 (33–75 x 4.1–6.2)μ

6–septate 7–septate	9 per cent	72 x 5.2μ (only a few measured)
8–septate 9–septate	8 per cent	80 x 5.3μ (only a few measured) 102 x 5.8μ (only a few measured, the largest 105 x 6.5μ)
11–septate 12–septate	4 per cent	91 x 5.8μ (only one measured) 101 x 5.7μ (only one measured)

On hard lima-bean agar, culture thirteen days old; conidia from thin pseudopionnotes:

Conidia: 5–septate, 9 per cent, 58 x 4.1 (53–62 x 3.9–4.4)μ
6–septate, 12 per cent, 78 x 4.3 (70–87 x 4.1–4.5)μ
7–septate, 19 per cent, 86 x 4.3 (78–96 x 4.1–4.5)μ
8–septate, 32 per cent, 93 x 4.4 (86–100 x 4.2–4.8)μ
9–septate, 17 per cent, 94 x 4.4 (90–100 x 4.2–4.7)μ
10–septate, 7 per cent, 94 x 4.4 (97–103 x 4.3–4.5)μ (only four measured)
11–septate, 2 per cent, 108 x 4.4μ (only one measured)
12–septate, 1 per cent, 110 x 4.4μ (only one measured)
13–septate, 1 per cent, 126 x 4.8μ (only one measured)

On wheat grain, culture fifteen days old; conidia from aërial mycelium:

Conidia: 0–septate, very rare
1–septate, very rare
2–septate, very rare, 26 x 3.1μ (only a few measured)
3–septate, 5 per cent
4–septate, 2.5 per cent
5–septate, 89 per cent, 60 x 4.2 (45–70 x 3.9–4.7)μ

6–septate 7–septate	3 per cent	75 x 4.2 (67–80 x 4.1–4.4)μ (only a few measured) 85 x 4.3μ (only a few measured)
8–septate 9–septate	0.5 per cent	81 x 4.6μ (only a few measured) 86 x 4.5μ (only a few measured, the longest 92 x 4.5μ)

On hard lima-bean agar, culture nine days old; conidia from pseudo pionnotes:

Conidia: 0–septate
1–septate } 1 per cent
2–septate
3–septate, 2 per cent, 45 x 4 (only a few measured)
5–septate, 40 per cent, 63 x 4.3 (43–76 x 3.5–4.7)μ
6–septate, 22 per cent, 72 x 4.4 (62–79 x 4–4.7)μ
7–septate, 18 per cent, 80 x 4.5 (63–88 x 4.3–4.8)μ
8–septate, 10 per cent, 87 x 4.7 (75–93 x 4.3–4.8)μ
9–septate, 5 per cent, 88 x 4.7μ (only two measured)
10–septate, 2 per cent, 90 x 4.8μ (only one measured)

On wheat grain, culture forty-six days old; conidia from aërial mycelium close to substratum:

Conidia: 0–septate, 3 per cent: arthrosporial, 15 x 4.1 (10–22 x 3.9–4.4)μ; sickle-shaped, 20 x 2.8 (16–22 x 2.6–3.5)μ
1–septate, 27 per cent
2–septate, 4 per cent } arthrosporial, 27 x 4.4 (20–38 x 3.9–5.3)μ
3–septate, 38 per cent
4–septate, 11 per cent
5–septate, 15 per cent, 53 x 3.9 (42–62 x 3.5–4.2)μ
6–septate, 2 per cent, 65 x 4.2 (54–70 x 3.9–5.2)μ
7– and 8–septate, rare, 73–81 x 4.1–4.8μ (only three measured)

Average of the above measurements:

Conidia: 0–septate, 0.6 per cent
1–septate, 4.6 per cent
2–septate, 2.2 per cent
3–septate, 14 per cent
4–septate, 4 per cent
5–septate, 40 per cent, 50 x 4.2μ
6– and 7–septate, 16.8 per cent, 76 x 4.6μ
8– and 9–septate, 14.4 per cent, 89 x 4.86μ
10– to 15–septate, 3.4 per cent, 103 x 4.9μ (the largest conidium found was 15–septate, 150 x 6μ)

22. *Fusarium anguioides* var. **caudatum** n. var. (Fig. 19; Pl. VI, fig. 9) Conidial type very much the same as that of *F. anguioides*, but 8- to 11-septate conidia very rare and the size for the same septation somewhat larger; conidia never in macroscopically observable thick pseudopionnotes as is often the case in *F. anguioides* when grown on various agars. Conidia of this fungus, even in comparatively young cultures, often produce on one end a long, usually unbranched, germ tube (see certain conidia in figure 19), which has never been observed in *F. anguioides*.

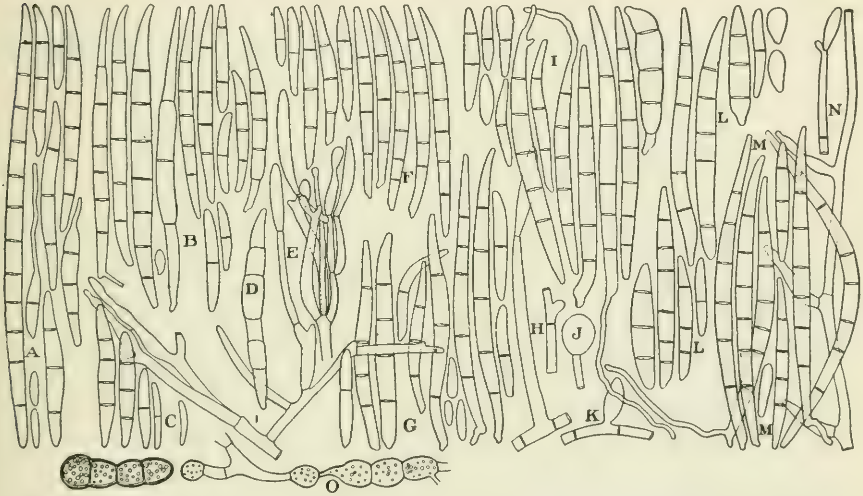

FIG. 19.—*Fusarium anguioides var. caudatum.* A, *Pseudopionnotal conidia from 10-days-old culture on slightly acidified hard potato agar;* B, *conidia from 61-days-old culture on red raspberry cane plug;* C, *typical,* D, *degenerated, conidia,* E, *conidiophores, from 67-days-old culture on potato stem plug;* F, *conidia from 15-days-old culture on wheat grain;* G, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar;* H, *conidiophores,* I, *pseudopionnotal conidia,* J, *chlamydospore-like mycelial swelling,* K, *conidiophores, from 11-days-old culture on medium potato agar;* L, *pseudopionnotal conidia from 16-days-old culture on hard lima-bean agar with 2 per cent glucose;* M, *pseudopionnotal conidia from 37-days-old culture on hard oat agar;* N, *conidiophores from 11-days-old culture on medium potato agar*

Hab. On rotted tubers of *Solanum tuberosum,* in association with *F. coeruleum,* Ithaca, New York.

Measurements of conidia from cultures on different media are as follows:

On red raspberry cane plug, culture sixty-one days old:

Conidia: 0– and 1–septate, 10 per cent
2– to 4–septate, 35 per cent
5–septate, 55 per cent, 56 x 4.2 (35–80 x 3.7–4.8)μ
6–septate, rare, the longest 85 x 5.2μ

On hard lima-bean agar, culture sixteen days old:

Conidia: 0–septate, 17 per cent, 13 x 2.7 (11–16 x 2.3–3.7)μ
1–septate, 18 per cent, 22 x 3.3 (18–28 x 3–4.4)μ, the broadest 33 x 6.1μ
2–septate, 12 per cent
3–septate, 40 per cent, 38.5 x 4 (29–45 x 3.5–4.7)μ, the broadest 33 x 6.1μ
4–septate, 3 per cent
5–septate, 7 per cent, 60 x 4.5 (52–75 x 4.1–5.4)μ
6–septate, 3 per cent, 67 x 5.2 (53–77 x 4.1–6.1)μ
7–septate, very few, 82 x 5.2μ (only one measured)
8–septate, few, 75 x 5.3 (72–88 x 5–5.6)μ (only two measured)
9–septate, very few, 94 x 5.2μ (only one measured)

On hard potato agar, culture eleven days old:

Conidia: 0–septate, 12 per cent, 14 x 3.9 (8.7–20 x 2.6–4.2)μ
1–septate, 11 per cent, 17 x 4.1μ (only four measured)
2–septate, 9 per cent
3–septate, 47 per cent, 40 x 4.3 (25–53 x 3.5–5.3)μ
4–septate, 8 per cent
5–septate, 12 per cent, 66 x 4.6 (50–77 x 4–6.1)μ

$$\left.\begin{array}{l}\text{6–septate,}\\ \text{7–septate,}\end{array}\right\}\text{ 1 per cent }\left\{\begin{array}{l}\text{79 x 5.3}\mu\text{ (only one measured)}\\ \text{85 x 5.2}\mu\text{ (only one measured)}\end{array}\right.$$

On wheat grain, culture fifteen days old:

Conidia: 0–septate, 4 per cent
1–septate, 40 per cent, 24 x 3.1 (15–27 x 2.7–3.6)μ
2–septate, 10 per cent
3–septate, 42 per cent, 43 x 3.8 (36–62 x 3.5–5.2)μ
4–septate, 2 per cent
5–septate, 2 per cent, 55 x 4.1 (43–62 x 4–4 5)μ
6–septate, few, 57 x 4.3μ (only one measured)
7–septate, very few, 79 x 4.3μ (only one measured)

On hard lima-bean agar, culture nine days old:

Conidia: 0–septate, 14 per cent
1–septate, 28 per cent
2–septate, 5 per cent
3–septate, 32 per cent, 44 x 3.6 (29–63 x 3–4)μ (exceptionally 4.8μ in diameter)
4–septate, 5 per cent
5–septate, 16 per cent, 65 x 3.9 (52–79 x 3.5–4.8)μ

Average of the above measurements:

Conidia: 0–septate, about 10.4 per cent, 13.5 x 3.3μ
1–septate, about 20.4 per cent, 21 x 3.5μ
2–septate, about 9.2 per cent
3–septate, about 35.2 per cent, 41 x 3.9μ
4–septate, about 5.6 per cent
5–septate, about 18.4 per cent, 60.4 x 4.26μ
6– and 7–septate, about 0.8 per cent, 82 x 4.9μ
8– and 9–septate, very rare, 85 x 5.2μ

23. **Fusarium arthrosporioides** n. sp. (Figs. 1, L to P, and 20; Pl. III, figs. 3 and 4; Pl. VII, fig. 11)

Conidia of three types: (1) elliptical, apically attenuate, slightly pedicellate, mostly 5–septate, 48 x 4.3 (45–55 x 4–4.7)μ; (2) arthrosporial, 0– to 3–(5–)septate, broad spindle-shaped, 3-septate measuring about 25 x 5.4 (19–33 x 4.7–6)μ; (3) sporotrichial, mostly 0–septate, 4.5 x 3.8 (2.9–9 x 2.6–8)μ. Type 1 is found mostly in pseudopionnotes, type 2 on aërial mycelium, and type 3 mainly in small, dense, bushlike clusters of conidiophores, often resembling sporodochia; no true chlamydospores; aërial mycelium from white to light tints of rose and salmon hues, uniform or covered with knotted, more or less large, areas; substratum from clay color to buckthorn brown and bright red (Plate III, fig. 4). The organism occupies an intermediate position between sections Arthrosporiella and Sporotrichiella.

Hab. In discolored tissues of tuber of *Solanum tuberosum*, Ireland.

The organism remained for a long time in pure culture, with well-developed aërial mycelium and with a bright red color (Plate III, fig. 4); the dominant type of conidia was sickle-shaped, 5-septate. During the last year — the second year of its cultivation — the development of aërial mycelium has become poorer, the color of the substratum has changed to clay color and buckthorn brown, and sporotrichic conidia are the commonest type. Whether the organism has changed only temporarily or not is as yet unknown.[43]

Latin description.—Conidiis tribus typis: (1) conidiis ellipticis, apice attenuatis, paulum pedicellatis, plerumque 5–septatis, 48 x 4.3 (45–55 x 4–4.7)μ; (2) conidiis arthrosporialibus, 0–3(5)–septatis, latis fusiformibus, 3–septatis circa 25 x 5.4 (19–33 x 4.7–6)μ; (3) conidiis sporotrichialibus, plerumque 0–septatis, 4.5 x 3.8 (2.9–9 x 2.6–8)μ. Typo primo plerumque in pseudopionnotibus, typo altero in aerio mycelio, typo tertio plerumque in parvis densis fruticosis conidiophoris uveosis, saepe similibus sporodochiis; nullis veris chlamydosporis; aerio mycelio ex albo pallide roseo "salmon color" (R), uniformi vel nodosas plus minusve magnas areas

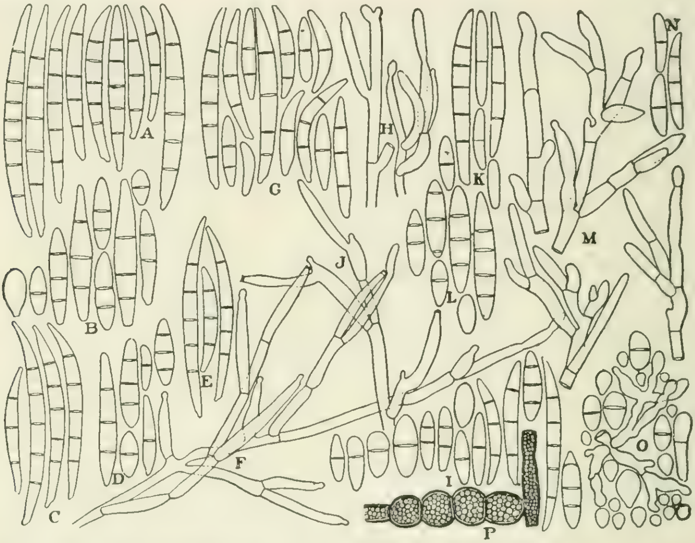

Fig. 20.—*Fusarium arthrosporioides.* A, *Normal pseudopionnotal conidia,* B, *arthrosporial conidia, from 14-days-old culture on hard lima-bean agar;* C, *sporodochial conidia from 64-days-old culture on hard lima-bean agar with 2 per cent glucose;* D, *arthrosporial,* E, *normal, conidia,* F, *conidiophores, from 11-days-old culture on medium potato agar;* G, *aërial conidia,* H, *conidiophores, from 11-days-old culture on slightly acidified hard potato agar;* I, *conidia and chlamydospores from 76-days-old culture on red raspberry cane plug;* J, *conidiophores from 11-days-old culture on medium potato agar;* K, *normal,* L, *arthrosporial, type of conidia,* M, *conidiophores, from aërial mycelium of 16-days-old culture on hard lima-bean agar with 2 per cent glucose;* N, *sickle-shaped conidia,* O, *arthrosporial and sporotrichial conidia and conidiophore of the sporodochial stage, from 36-days-old culture on rye grain;* P, *chlamydospores*

exhibente; substrato ex argillaceo "buckthorn brown" (R) vel nitide rubro (Tab. III, fig. 4). Fungo locum medium inter Sectiones Arthrosporiellam et Sporotrichiellam obtinente.

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[43] In this connection see last paragraph in description of *F. biforme* (page 168).

Hab. In textibus decoloratis Solani tuberosi tuberum, Hibernia.

Measurements on different media are as follows:

On slightly acidified hard potato agar, culture eleven days old; conidia from aërial mycelium:

Conidia: 0–septate, 0.5 per cent, 12 x 2.7μ
1–septate, 15.5 per cent, 25 x 3.8 (17–30 x 2.6–4.7)μ
2–septate, 3 per cent, 27 x 4μ
3–septate, 77.5 per cent, 35 x 3.5 (22–48 x 3–4.4)μ
4–septate, 3 per cent, 43 x 4.2 (35–50 x 3.5–4.8)μ
5–septate, 0.5 per cent, 47 x 4.4μ (only a few measured)

On hard lima-bean agar with 2 per cent glucose, culture sixteen days old:

(1) Sickle-shaped conidia
- 0–septate, 4.7 per cent, 10 x 2.6μ (only a few measured)
- 1–septate, 4.7 per cent, 19 x 3.1 (14–24 x 2.9–3.5)μ
- 2–septate, few, 28 x 3.3μ (only a few measured)
- 3–septate, 5.9 per cent, 34 x 4 (31–40 x 3.3–4.7)μ
- 4–septate, 0.5 per cent, 43 x 4.3 (35–44 x 4.1–4.7)μ
- 5–septate, 0.6 per cent, 47 x 4.4 (43–54 x 4.1–4.8)μ

(2) Arthrosporial conidia
- 0–septate, 13 per cent, 8.5 x 4.3 (6–12 x 3.5–6.4)μ
- 1–septate, 23.5 per cent, 15 x 4.4 (9–20 x 3.5–5.3)μ
- 2–septate, 11.7 per cent, 18 x 5.2 (13–35 x 4.7–6)μ
- 3–septate, 35.4 per cent, 25 x 5.4 (19–33 x 4.7–6)μ
- 4–septate, few, 39 x 6 (33–47 x 5–7)μ
- 5–septate, few, 46 x 5.7 (42–56 x 5.2–6)μ

The largest conidium observed was 8–septate (exceptionally close-septate), and measured 58 x 6.5μ

On potato stem plug, culture one hundred and seven days old:
Conidia: 1–septate, 52 per cent, about 13 x 3.5μ
2–septate, 7 per cent
3–septate, 31 per cent, 34 x 3.4μ
4–septate, 3 per cent, 43 x 3.6μ
5–septate, 7 per cent, 45 x 4 (40–50 x 3.4–4.3)μ

On hard lima-bean agar, culture sixty-four days old; conidia from a sporodochium:
Conidia: 1–septate, 1 per cent
3–septate, 12 per cent, 26 x 3.4 (22–44 x 3–3.6)μ
4–septate, 5 per cent, 43 x 3.9μ (only two measured)
5–septate, 81 per cent, 48 x 4 (43–52 x 3.5–4.3)μ
6–septate, 1 per cent, about 50 x 4.1μ (only two measured)

On hard lima-bean agar, culture fourteen days old; conidia from a dense mycelial growth at the bottom of the slant:
Conidia: 0–septate, 1.5 per cent, 13 x 4.8μ (only three measured)
1–septate, 10 per cent, 20 x 5.2 (9–27 x 4.1–7)μ
2–septate, 4 per cent, 22 x 8.3μ (only three measured)
3–septate, 38 per cent, 28 x 5.2 (19–39 x 4.3–5.9)μ
4–septate, 4 per cent
5–septate, 37 per cent, 55 x 4.7 (43–70 x 4.4–5.3)μ
6–septate, 4 per cent, 61 x 5 (54–70 x 4.8–5.3)μ
7–septate, 1.5 per cent, 69 x 5 (64–79 x 4.7–5.3)μ

On rye grain, culture thirty-six days old; conidia from a sporodochium, of sporotrichial type:
Conidia: 0–septate, 97 per cent, 4.5 x 3.8 (2.9–9 x 2.6–8)μ
1–septate, 3 per cent, 10.2 x 5.6 (8.5–14 x 4.2–6.3)μ
2–septate, rare
3–septate, rare

Average of the above measurements:
Conidia: 0–septate, 17 per cent $\begin{cases} \text{4.5 x 3.8μ (sporotrichial form)} \\ \text{12.1 x 3.8μ (arthrosporial form)} \end{cases}$
1–septate, 18 per cent, 17 x 14.2μ
2–septate, 6 per cent
3–septate, 33 per cent, 25 x 5.4μ (arthrosporial form)

4–septate, 3 per cent
5–septate, 21 per cent, 48 x 4.3μ (sickle-shaped)
6–septate, 1 per cent, 55.5 x 4.55μ (sickle-shaped)
7–septate, 0.5 per cent, 69 x 5μ (sickle-shaped)

In the case of this organism, the average percentage of septation of conidia is of no value because of the great diversity of the forms occurring. Therefore it is a safer basis to consider each stage (sporodochial and from aërial mycelium) by itself separately.

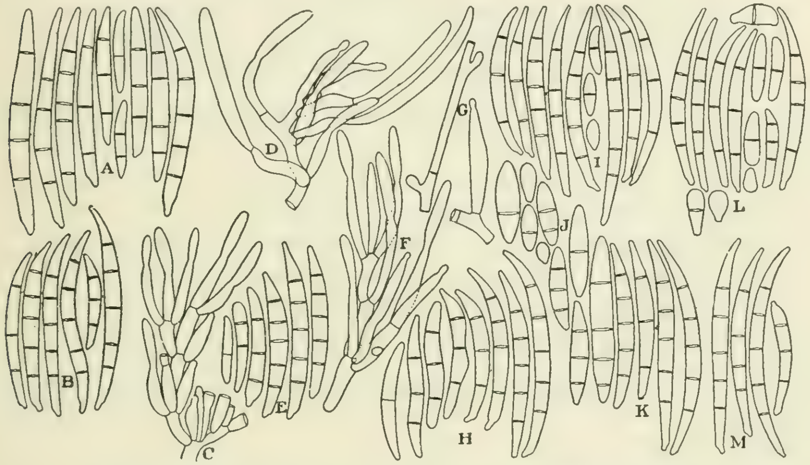

Fig. 21.—*Fusarium arthrosporioides var. asporotrichius.* A, *Aërial conidia from 7-days-old culture on hard lima-bean agar with 2 per cent glucose;* B, *sporodochial conidia from 82-days-old culture on potato tuber plug;* C, D, *conidiophores, from 11-days-old culture on slightly acidified hard potato agar;* E, *aërial conidia from 25-days-old culture on potato tuber plug;* F *and* G, *conidiophores,* H, *pseudopionnotal conidia, from 11-days-old culture on slightly acidified hard potato agar;* I, *conidia from 68-days-old culture on red raspberry cane plug;* J, *arthrosporium-like,* K, *normal, conidia from aërial mycelium from 14-days-old culture on hard lima-bean agar;* L, *aërial conidia from 36-days-old culture on rye grain;* M, *sporodochial conidia from 14-days-old culture on hard lima-bean agar*

24. *Fusarium arthrosporioides* var. **asporotrichius** n. var. (Figs. 1, R and S, and 21; Pl. VII, fig. 9)

This fungus is very much the same as *F. arthrosporioides,* but differs from the latter by typical absence of sporotrichial sporodochia and by more pronounced and common production of from medium to large (up to 3 millimeters in diameter) sporodochia with sickle-shaped, 5–septate conidia, 51.2 x 4.3 (46–59 x 4.15–4.7)μ, as dominant type.

Hab. On rotted tubers of *Solanum tuberosum* in association with *F. Solani* and *F. coeruleum*, New York State.

Measurements on different media are as follows:

On slightly acidified hard potato agar, culture eleven days old, conidia from pseudopionnotes:

Conidia: 0–septate, 0.8 per cent
1–septate, 3.4 per cent, 20 x 3.3μ
2–septate, 2.5 per cent, 30 x 3.8μ
3–septate, 49 per cent, 40 x 4 (30–56 x 3–4.7)μ
4–septate, 21.8 per cent, 50 x 4.3 (43–60 x 3.9–4.5)μ
5–septate, 22.5 per cent, 53.5 x 4.4 (49–62 x 4–4.7)μ

On red raspberry cane plug, culture sixty-eight days old; conidia from a sporodochium:

Conidia: 1–septate, very rare
2–septate, very rare
3–septate, 9 per cent, 41 x 3.7 (21–48 x 3.3–4.4)μ
4–septate, 24 per cent, 46 x 4.1 (36–60 x 3.8–4.7)μ
5–septate, 67 per cent, 51 x 4.2 (42–62 x 3.9–4.7)μ
6–septate, rare, 62–70 x 4.1–4.8μ (only a few measured)

On potato tuber plug, culture eighty-two days old; conidia from a sporodochium:

Conidia: 1–septate, rare
2–septate, rare
3–septate, 6 per cent, 36 x 3.8 (25–42 x 3.5–4.1)μ
4–septate, 17 per cent
5–septate, 77 per cent, 49 x 4.2 (43–55 x 3.5–4.7)μ

On hard lima-bean agar, culture seven days old; conidia from aërial mycelium:

Conidia: 0–septate, 3 per cent
1–septate, 8 per cent
2–septate, 8 per cent
3–septate, 65 per cent, 39 x 4.1 (31–67 x 3.9–5.7)μ, the longest 68 x 4.4μ
4–septate, 14 per cent, 49 x 4.7 (44–60 x 4.2–5.3)μ
5–septate, 2 per cent, 59 x 4.8 (52–63 x 4.3–5.7)μ

On potato tuber plug, culture twenty-five days old; conidia from aërial mycelium:

Conidia: 0–septate, rare, about 16 x 2.5μ
1–septate, 7 per cent, 22 x 3.7μ (only three measured)
2–septate, 6 per cent, 27 x 4.1μ (only three measured)
3–septate, 40 per cent, 35 x 4.3 (29–45 x 3.8–4.8)μ
4–septate, 28 per cent, 43 x 4.7 (35 x 49 x 4.1–5.2)μ, the broadest 51 x 5.9μ
5–septate, 19 per cent, 45 x 4.7 (40–53 x 4.3–4.9)μ
6–septate, only one observed, 78 x 5.2μ

On whole steamed potato tuber, culture forty-six days old:

(1) Conidia from a medium large (2 millimeters in diameter) sporodochium

Conidia: 1–septate, 2.5 per cent, about 16 x 3μ
2–septate, 2.5 per cent, about 28 x 3.5 (20–40 x 3.3–3.8)μ
3–septate, 31 per cent, 38.5 x 3.7 (19–44 x 3–4.1)μ
4–septate, 34 per cent, 45 x 4μ (only three measured)
5–septate, 30 per cent, 48 x 4.15 (44–53 x 3.9–4.7)μ
6–septate, very rare, 61 x 4.3μ (only one measured)

(2) Conidia from a small sporodochium

Conidia: 1–septate, 2 per cent
2–septate, rare
3–septate, 51 per cent, 40 x 3.9 (35–43 x 3–4.1)μ
4–septate, 24 per cent, 42 x 4.1 (36–50 x 3.5–4.8)μ
5–septate, 23 per cent, 46 x 4.2 (42–50 x 4–4.5)μ

On medium potato agar, culture eleven days old; conidia from pseudopionnotes:

Conidia: 3–septate, 13 per cent, 39 x 4.3 (28–48 x 3.5–4.8)μ
4–septate, 8 per cent
5–septate, 79 per cent, 48 x 4.6 (41–61 x 3.8–4.9)μ

On hard lima-bean agar, culture fourteen days old:

(1) Conidia from aërial mycelium

Conidia: 0–septate, 6 per cent, 11 x 4.6 (6–18 x 4–5.3)μ
1–septate, 13 per cent, 18 x 5 (10–25 x 4.1–5.5)μ

2–septate, 10 per cent, 24 x 5.1 (20–30 x 4.3–5.5)μ (only three measured)
3–septate, 39 per cent, 31 x 5.2 (22–43 x 4.1–6.2)μ
4–septate, 7 per cent
5–septate, 25 per cent, 56 x 4.7 (42–70 x 4–5.9)μ
6–septate, very few, 63 x 4.9μ (only one measured)

(2) Conidia from a sporodochium
Conidia: 3–septate, about 5 per cent, 36 x 3.1μ (only a few measured)
4–septate, about 5 per cent
5–septate, about 90 per cent, 58 x 4 (52–65 x 3.5–4.8)μ

On rye grain, culture thirty-six days old:
Conidia: 0–septate, 3 per cent
1–septate, 6 per cent
2–septate, 2 per cent
3–septate, 55 per cent, 38 x 3.4 (27–50 x 3–4.4)μ
4–septate, 20 per cent
5–septate, 14 per cent, 50 x 3.6 (43–60 x 3.1–4.4)μ

Average of the above measurements:
Conidia: 0–septate, 1 per cent
1–septate, 3 per cent
2–septate, 3 per cent
3–septate, 33 per cent
4–septate, 19 per cent
5–septate, 41 per cent, 51.2 x 4.3μ
6–septate, very rare

25. *Fusarium diplosporum* Cke. et Ell.

Cf. Cooke, M. C., and Ellis, J. B., Grevillea **7**:38. 1878. Saccardo, Syll. Fung. **4**:701. 1886.

The following description is from Saccardo:

" Roseum, pulvinatum; conidiis aliis fusiformibus, utrinque acutis, arcuatis, nucleatis, demum leniter 3–septatis 40μ long., aliis ellipticis, uniseptatis 18 x 8μ.

" Hab. In caulibus Solani tuberosi, New Jersey, Amer. bor."

The abbreviated, arthrosporial, uniseptate, conidia, 18 x 8μ in size, are so uncommon that there is no doubt in the writer's mind that the organism could easily be identified when found, and thus must be recognized as a good species. The organism was not isolated by the writer.

VII. SECTION SPOROTRICHIELLA Wr. (Fig. 1Q). Wollenweber, H. W., Maine Agr. Exp. Sta., Bul. 219:256. 1913

Fusaria of this section have from pyriform to nearly globular, mostly 0–septate, microconidia. Typical sickle-shaped, septate conidia always present, at least in young artificial cultures.

Only one species of this section is presented here. This species was twice isolated from rotted potato tubers, and thus, at least in a way, may be considered as of more or less common occurrence on this substratum. There are many other Fusaria of this section reported on such substrata as corn, carnations, apples, and others, a number of which are discussed by Lewis (1913). None of the species of this section mentioned by him, however, seem to be identical with the one here reported.

Lewis (1913:257) reports that the following organisms were isolated also from potatoes: (1) *F. Poae* (Peck) Wr., (2) *F. Solani* (Mart.) Ap. et Wr., (3) *F. conglutinans* Wr., (4) *F. Helianthi* Sacc. var., (5) *F. pirinum* (Fries) Sacc., and (6) *F. orthoceras* Ap. et Wr.

Numbers 2 and 6 are described in this paper; numbers 1 and 4 are closely related to *F. sporotrichioides* n. sp., and belong to section Sporotrichiella; number 3 belongs to section Elegans and differs from *F. orthoceras* by absence of red-wine color on rice (see Wollenweber, 1913 a:30); number 5 may belong to section Arthrosporiella.

No technical description, except results of inoculations — for potatoes always negative — and certain characters of color and of colony growth, is given, and thus a proper identification is rendered impracticable.

26. **Fusarium sporotrichioides** n. sp. (Figs. 1Q and 22; Pl. III, fig. 1)

Conidia scattered in aërial mycelium or in pseudopionnotes and distinct sporodochia; of diverse type, ranging from unicellular, more or less pyriform, microconidia of sporotrichial form, to sickle-shaped, 3– to 10–septate, apically pointed, pedicellate, macroconidia; 0–septate, sporotrichial conidia average 10.5 x 6 (9.5–11.4 x 5.6–6.5)μ; sickle-shaped, 3–septate conidia average 30.4 x 3.8μ, and 5-septate average 50.5 x 4.3μ; conidia of pseudopionnotes stage resemble those of *F. anguioides*; chlamydospores often present, intercalary, commonly in small clusters;

color of aërial mycelium and of substratum from white to clay (Pl. III, fig. 1) and pink, similar to the color of *F. bullatum* var. *roseum* (Pl. III, fig. 2).

Hab. On rotted tubers of *Solanum tuberosum*, together with *F. Solani* and *F. oxysporum*, New York State.

Latin description.—Conidiis in aerio mycelio sparsis vel in pseudopionnotibus et sporodochiis distinctis; typis variis: interdum microconidiis unicellularibus, plus minusve pyriformibus, sporotrichialibus; interdum macroconidiis falciformibus, 3–10–septatis, apice acutis, pedicellatis; conidiis 0–septatis sporotrichialibus plerumque 10.5 x 6 (9.5–11.4 x 5.6–6.5)μ; falciformibus, 3–septatis 30.4 x 3.8μ, et 5–septatis 50.5 x 4.3μ; conidiis pseudopionnotum gradu similibus conidiis F. anguioidum; chlamydosporis saepe visis, intercalaribus, plerumque minutis uveosis; aerio mycelio substratoque ex albo argillaceo vel rubello, simili colore F. bullati rosei.

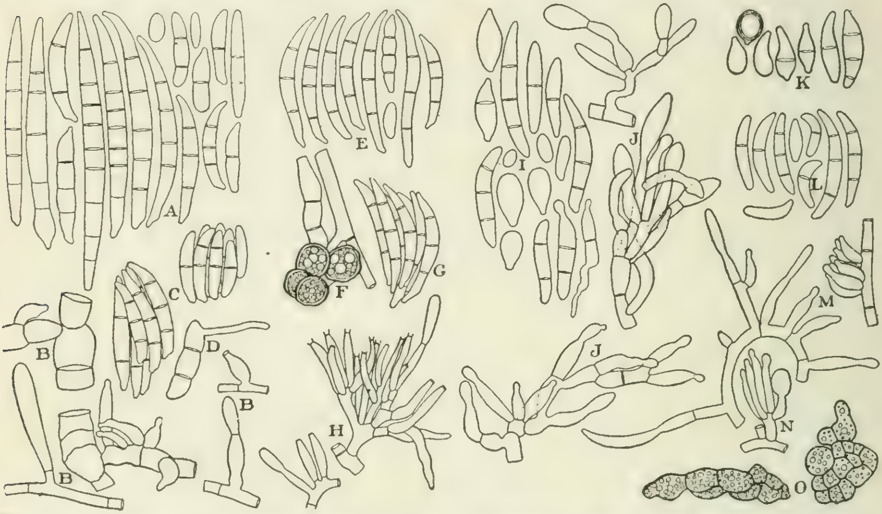

FIG. 22.—*Fusarium sporotrichioides.* A, *Pseudopionnotal conidia,* B, *various simple conidiophores,* C, *balls of conidia,* D, *germinating conidium, from 5-days-old culture on hard potato agar;* E, *sporodochial conidia,* F, *chlamydospores,* G, *ball of conidia,* H, *compound conidiophores, from 86-days-old culture on hard potato agar;* I, *sporotrichial conidia (some germinating),* J, *conidiophores, from aërial mycelium of 16-days-old culture on wheat grain;* K, *typical, thick-walled sporotrichial conidia,* L, *sickle-shaped conidia,* M, *conidiophores producing sickle-shaped conidia,* N, *conidiophore producing sporotrichial conidia,* O, *plectenchymic stroma, from 52-days-old culture on red raspberry cane plug*

Hab. In tuberibus putridis Solani tuberosi una cum F. Solani et F. oxysporo, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture five days old; conidia from pseudopionnotes:

Conidia: 0–septate, 2 per cent
1–septate, 10.5 per cent, 19 x 3.5μ
2–septate, 5.5 per cent, 28 x 3.7μ
3–septate, 37 per cent, 32.5 x 4.2 (24–50 x 3.9–4.7)μ
4–septate, 6 per cent, 45 x 4.2μ
5–septate, 33 per cent, 48 x 4.5 (40–62 x 3.9–5.3)μ
6– to 10–septate, 6 per cent, 50–90 x 5–6.1μ

On hard potato agar, culture eighty-six days old; conidia from a small sporodochium, semi-dry:

Conidia: 3–septate, nearly 100 per cent, 29.5 x 3.4 (20–41 x 3–4)μ

On red raspberry cane plug, culture fifty-two days old:

(1) Conidia of sporotrichial form
Conidia: 0–septate, 97 per cent, 9.5 x 6.5 (7–12 x 4–7.5)μ
1–septate, 3 per cent, 13.5 x 5.2 (10–15 x 4.8–6)μ
2–septate, rare
3–septate, exceptional, one measured, 21 x 5.2μ

(2) Conidia of sickle-shaped form
Conidia: 0–septate, 35 per cent, 11 x 2.9 (10–13 x 2.5–3.1)μ
1–septate, 60 per cent, 15.5 x 3.2 (12–22 x 2.9–3.5)μ
2–septate, very rare
3–septate, 5 per cent, 25 x 3.5 (20–28 x 3–4)μ

On hard lima-bean agar with 2 per cent glucose, culture 10 days old; conidia from a pseudopionnotes:

Conidia: 0–septate, 5 per cent, 10 x 3 (5.2–16 x 2.1–3.7)μ
1–septate, 15 per cent, 22 x 3.3 (16–28 x 3–3.5)μ
2–septate, 2 per cent
3–septate, 22 per cent, 33 x 3.8 (27–49 x 3.5–4.8)μ
4–septate, 23 per cent, 45 x 4 (38–49 x 3.9–4.1)μ
5–septate, 30 per cent, 51 x 4.1 (41–62 x 3.5–4.7)μ

6–septate, 1.5 per cent, 65 x 4.1 (60–80 x 3.7–4.4)μ (only four measured)
7–septate, 1.5 per cent, 67 x 4.5 (62–72 x 4.1–5.3)μ (only four measured)

On wheat grain culture sixteen days old; conidia from aërial mycelium:
(1) Sporotrichial form
Conidia: 0–septate, 70 per cent, 11.4 x 5.6 (8–16 x 4–6.5)μ
1–septate, 30 per cent, 14 x 5.6 (11–17 x 4.8–6)μ
(2) Sickle-shaped form
Conidia: 0–septate, 9 per cent, 10 x 3.2μ
1–septate, 68 per cent, 20 x 3.8 (12–24 x 3–4.2)μ
2–septate, 5 per cent
3–septate, 18 per cent, 32 x 4.3 (24–44 x 3.9–4.7)μ

Average of the above measurements:
(1) Sporotrichial form
Conidia: 0–septate, 83 per cent, 10.5 x 6μ
1–septate, 17 per cent, 14 x 5.4μ
2– and 3–septate, rare to very rare
(2) Sickle-shaped form
Conidia: 0–septate, 10 per cent
1–septate, 31 per cent, 10 x 3.1μ
2–septate, 2 per cent
3–septate, 36 per cent, 30.4 x 3.8μ
4–septate, 6 per cent
5–septate, 13 per cent, 50.5 x 4.3μ
6– to 10–septate, 2 per cent, about 67 x 4.5μ

VIII. Section **FERRUGINOSUM** n. sec.

Conidia mostly of an intermediate type, between that of section Gibbosum and section Roseum, 3– to 7–septate; intercalary chlamydospores typically present. Substratum and basal layer of aërial mycelium varying from white when young to different hues of red at maturity.

27. **Fusarium arcuosporum** n. sp. (Figs. $1B_1$ and 23; Pl. II, figs. 7 and 8; Pl. VI, fig. 10)

Conidia very gradually pointed toward apex, distinctly and often prominently pedicellate, typically much arcuate, 5-septate, 49.2 x 4 (42–54 x 3.6–4.2)μ, also often from 3- to 7-septate; single on aërial conidiophores and in from small to medium (up to 1.5 millimeters in diameter) sporodochia, the latter sometimes converging into pseudopionnotes; intercalary chlamydospores in mycelial threads typically present, though not numerous, and single or only in small clusters of from two to four cells; aërial mycelium usually well developed, uniform, white at first, then, on substrata poor in glucose, of different tints of pink with the substratum of a pomegranate color of different density; on potato agar rich in glucose, color as shown in Plate II, figures 7 and 8.

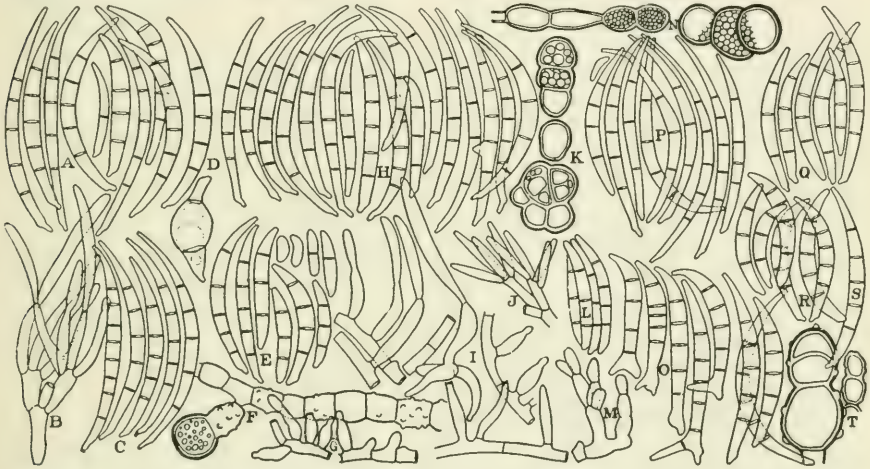

FIG. 23.—*Fusarium arcuosporum.* A, *Sporodochial conidia,* B, *conidiophore, from 20-days-old culture on hard lima-bean agar with 2 per cent glucose;* C, *conidia from small aërial sporodochium from 49-days-old culture on whole steamed potato tuber;* D, *typical conidium (the long one) and abnormal pseudopionnotal conidium from 11-days-old hard potato agar;* E, *aërial conidia,* F, *chlamydospores,* G, *conidiophores, from 75-days-old culture on red raspberry cane plug;* H, *pseudopionnotal conidia,* I, *conidiophores, from 12-days-old culture on slightly acidified hard potato agar;* J, *conidiophore,* K, *chlamydospores,* L, *conidial ball,* M, *conidiophores, from aërial mycelium from 75-days-old culture on red raspberry cane plug;* N, *chlamydospores from 173-days-old culture on corn agar;* O, *peculiarly branched,* P, *normal, pseudopionnotal conidia from 14-days-old culture on hard lima-bean agar;* Q, *conidia from aërial sporodochium of 107-days-old culture on potato stem plug;* R, *conidia from 109-days-old culture on rye straw;* S, *typical sporodochial conidium from 24-days-old culture on potato tuber plug;* T, *chlamydospores from 109-days-old culture on rye straw*

Hab. On rotted tubers of *Solanum tuberosum,* in association with *F. anguioides,* Castile, New York.

Latin description.—Conidiis maxime gradatim in apicem acutis, distincte vel saepe insignite pedicellatis, typice magnopere arcuatis, 5–septatis, 49.2 x 4 (42–54 x 3.6–4.2)μ, etiam saepe 3–7–septatis; continuis in aeriis conidiophoris vel minutis mediocribusve (usque ad $\frac{1}{5}$ mm. diam.) sporodochiis in pseudopionnotes interdum vergentibus; chlamydosporis intercalaribus sed non plurimis, continuis vel tantum minutis uveosis, 1–3–septatis; aerio mycelio fere plene maturo, uniformi, primum albo, dein, in substratis parum glucosis, rubello varie tincto, substrato "pomegranate" (R); in Solani tuberosi agare perglucoso, colore Tab. II, figg. 7, 8, exhibito.

Hab. In tuberibus putridis Solani tuberosi una cum F. anguioide, Castile, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture twelve days old; conidia from aërial mycelium:

Conidia: 0– and 1–septate, rare, immature
3–septate, 17 per cent, 43 x 3.5 (35–56 x 3–4.1)μ
4–septate, 19 per cent, 49 x 3.5 (38–59 x 3–4.1)μ
5–septate, 64 per cent, 54 x 3.7 (40–74 x 3–4.4)μ

On red raspberry cane plug, culture seventy-five days old; conidia from aërial mycelium:

Conidia: 3–septate, 10 per cent, 45 x 3.4 (28–51 x 3–4)μ
4–septate, 30 per cent, 50 x 3.5 (36–62 x 3–4.3)μ
5–septate, 60 per cent, 53 x 3.6 (44–62 x 3.5–4)μ

On hard lima-bean agar with 2 per cent glucose, culture seven days old; conidia from aërial mycelium:

Conidia: 3–septate, 30 per cent } (granulation of the protoplasm was too
4–septate, 15 per cent } great to clearly distinguish septation)
5–septate, 55 per cent, 53 x 3.8 (45–62 x 3.4–4.4)μ

On hard lima-bean agar with 2 per cent glucose, culture twenty days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare
1–septate, rare
4–septate, 4 per cent, 47 x 4.2μ (only two measured)
5–septate, 95 per cent, 54 x 4 (47–63 x 3.5–4.8)μ
6–septate, 1 per cent, 52 x 4.1μ (only two measured)

On potato tuber plug, culture twenty-four days old; conidia from small sporodochia:

Conidia: 1–septate, 1 per cent, about 14 x 3.5μ (only one measured)
3–septate, 10 per cent, 35 x 3.6 (27–43 x 3–4.4)μ
4–septate, 12 per cent
5–septate, 77 per cent, 45 x 4 (35–51 x 3.3–4.8)μ
6– and 7–septate, rare, 52 x 4.5μ (only two measured)

On potato stem plug, culture one hundred and seven days old; conidia from aërial mycelium:

Conidia: 0–septate, rare
1–septate, 2 per cent
2–septate, rare
3–septate, 50 per cent, 31 x 3.7μ
4–septate, 25 per cent, 40 x 4μ
5–septate, 23 per cent, 42 x 4.2 (35–48 x 3.9–4.7)μ

On whole steamed potato tubers, culture forty-nine days old:

(1) Conidia from numerous small sporodochia, close to the substratum

Conidia: 3–septate, 6 per cent, about 30 x 3.5 (only a few spores measured)
4–septate, 6 per cent
5–septate, 80 per cent, 46 x 4.1 (40–53 x 3.9–4.1)μ
6–septate, 5 per cent, 54 x 4.1 (42–65 x 4–4.7)μ
7–septate, 3 per cent, 59 x 4.2 (48–65 x 4.1–4.5)μ
10–septate, only one was observed, 58 x 4.3μ (the largest)

(2) Conidia from small sporodochia, in aërial mycelium

Conidia: 3–septate, rare
4–septate, rare
5–septate, nearly 100 per cent, 51 x 4 (45–55 x 3.6–4.3)μ
6–septate, rare

On medium soft agar, culture eleven days old; conidia from aërial mycelium close to substratum:

Conidia: 1–septate, 2 per cent, 24 x 3.2 (only a few measured)
3–septate, 8 per cent, 42 x 4μ (only a few measured)
4–septate, 10 per cent
5–septate, 77 per cent, 45 x 4.1 (41–62 x 3.1–4.8)μ
6–septate, 3 per cent, 49 x 4.3μ (only a few measured)

On hard lima-bean agar, culture fourteen days old; conidia from minute converging sporodochia close to substratum:

Conidia: 3–septate, 3 per cent
4–septate, 1 per cent
5–septate, 95 per cent, 49 x 4.2 (41–63 x 3.5–5.3)μ
6–septate, 1 per cent, 54 x 4.7μ (only a few measured)

Average of the above measurements:

Conidia: 0– to 2–septate, very rare
3–septate, 13 per cent, 38 x 3.5μ
4–septate, 13.5 per cent
5–septate, 72.5 per cent, 49.2 x 4μ
6– and 7–septate, 1 per cent, 53.6 x 4.3μ

The organism occupies an intermediate position between section Roseum and section Ferruginosum; its type of conidia is that of section Roseum, while because of its true chlamydospores of only an intercalary kind it belongs to the section Ferruginosum.

An especially striking peculiarity of *F. arcuosporum* is the frequent occurrence, especially in young cultures on various agars, of conidia with more or less distinct branches and knobs (Fig. 23 o).

28. **Fusarium ferruginosum** n. sp. (Figs. 1, H to J, and 24; Pl. III, figs. 9 and 10; Pl. VI, fig. 2)

Conidia well developed, in from small to medium-sized (up to 2 millimeters in diameter) sporodochia or in pseudopionnotes, very gradually pointed toward apex, distinctly pedicellate, more or less strongly arcuate, and broader in the middle or in the lower third of their length, typically 3– to 5–septate; 5–septate average 45.3 x 4.2 (28–53 x 4.1–4.2)μ; conidia from aërial mycelium — sometimes also from sporodochia produced on very old agar cultures — typically 3–septate, 30.8 x 3.8μ, mostly apedicellate; conidia from nearly white to pale pink buff and deep vinaceous in color; intercalary chlamydospores in mycelium always present, often very numerous, in long chains and large clusters; aërial mycelium always well developed, high, often very dense, white at first changing to pink and then ferruginous when mature, the last-named color being due to production of great masses of chlamydospores; color of substratum, on potato agar rich in glucose, at first pink, then ochraceous tawny, and finally ferruginous and Hay's russet.

Hab. On rotted tubers of *Solanum tuberosum*, Long Island, New York, on *Lycopersicum esculentum*, Virginia, and on *Panax quinquefolium*, New York State.

Latin description.—Conidiis plene maturis—sporodochiis minutis vel mediocribus (usque ad 2 mm. diam.) vel pseudopionnotibus — maxime gradatim in apicem acutis, distincte pedicellatis, plus minusve valide arcuatis, latioribus in medio vel in inferiore tertio longitudinis, typice 3–5-septatis; 5-septatis plerumque 45.3 x 4.2 (28–53 x 4.1–4.2) μ; conidiis in aerio mycelio — interdum etiam ex sporodochiis in vetustissimis agaris culturis crescentibus — typice 3-septatis, 30.8 x 3.8μ, plerumque apedicellatis; conidiis ex albido pallide rubellis, gilvis atque vinaceosis; chlamydosporis intercalaribus semper in mycelio praesentibus, saepe plurimis, longis catenulatis longis uveosisque; aerio mycelio semper plene maturo, alto, semper densissimo, primum albo, dein rubello demum ferrugineo, quem colorem magnae moles chlamydosporum ortae efficiunt; substrato in agare Solani tuberosi glucoso, primum rubello, dein "ochraceous tawny" (R), demum ferrugineo et "Hay's russet" (R).

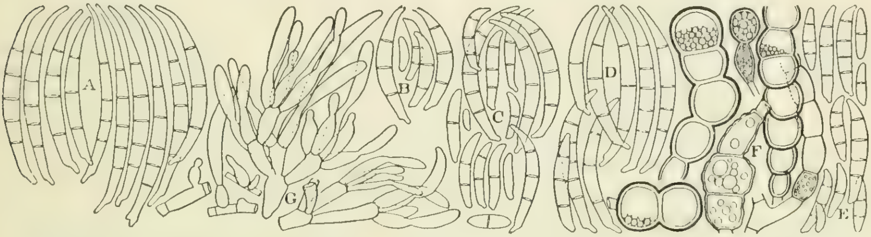

FIG. 24.— *Fusarium ferruginosum.* A, *Pseudopionnotal conidia from 10-days-old culture on hard lima-bean agar;* B, *sporodochial conidia from 130-days-old culture on hard lima-bean agar with 2 per cent glucose;* C, *aërial conidia from 14-days-old culture on slightly acidified hard potato agar;* D, *pseudopionnotal conidia from 3-days-old culture on hard lima-bean agar;* E, *aërial conidia,* F, *chlamydospores, from 35-days-old culture on corn meal;* G, *conidiophores from 3-days-old culture on hard lima-bean agar*

Hab. In tuberibus putridis Solani tuberosi, Long Island, New York, in Lycopersico esculento, Virginia, et in Panaci quinquefolio, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On hard lima-bean agar with 2 per cent glucose, culture one hundred and thirty days old; conidia from small sporodochia produced after the culture was over eighty days old:

Conidia: 0–septate, very rare, 9 x 2.8μ
1–septate, 4 per cent, 12 x 3.2μ
2–septate, 3 per cent, 17 x 3.5μ
3–septate, 93 per cent, 27.3 x 4.1 (17–33 x 3.8–4.4)μ

On hard potato agar with 10 per cent glucose, culture seven days old in poured plate:

(1) Chlamydospores
0–septate, 10 x 9.5 (7–12.5 x 7–11.5)μ
1–septate, 16 x 11μ
(2) Conidia from aërial mycelium
Conidia: 1–septate, 1 per cent
2–septate, 1 per cent
3–septate, 88 per cent, 32 x 3.7 (25–40 x 3.1–4.2)μ
4–septate, 6 per cent
5–septate, 4 per cent, 38 x 4.1 (35–42 x 3.9–4.4)μ

On hard lima-bean agar, culture three days old; conidia from pseudopionnotes (near the center of inoculation):

Conidia: 3–septate, 66 per cent, 36 x 3.9 (30–41 x 3.5–4.3)μ
4–septate, 20 per cent
5–septate, 14 per cent, 45 x 4.2 (42–56 x 3.9–4.7)μ

On corn meal agar, culture thirty-five days old:

(1) Chlamydospores typically in chains, 8.5 (5.8–15)μ in diameter
(2) Conidia from aërial mycelium
Conidia: 0–septate, 20 per cent, 8.8 x 2.6 (7–12 x 2.4–3)μ
1–septate, 46 per cent, 12.8 x 2.9 (9–19 x 2.5–3.1)μ
2–septate, 6 per cent
3–septate, 26 per cent, 20.4 x 3.4 (17–30 x 3–3.8)μ
4–septate, 2 per cent, 26.7 x 3.9 (24–29 x 3.7–4)μ

On hard lima-bean agar, culture ten days old; conidia from pseudopionnotes:

Conidia: 3–septate, 8 per cent, 41 x 3.9μ
4–septate, 12 per cent, 45.5 x 4μ

5–septate, 80 per cent, 53 x 4.2 (50–58 x 4.1–4.4)μ, the largest observed being 65 x 4.1μ

On slightly acidified hard potato agar, culture fourteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 26 per cent, 10 x 2.5 (8–14 x 1.7–3.5)μ
1–septate, 41.5 per cent, 17.3 x 3 (14–20 x 2.5–3.5)μ
2–septate, 10 per cent, 19.5 x 3.2 (15–31 x 2.9–4)μ
3–septate, 17.5 per cent, 28 x 3.6 (19–42 x 2.9–4.7)μ
4–septate, 3 per cent
5–septate, 2 per cent } 38 x 4.2 (30–45 x 3.9–5.2)μ

Average of the above measurements:

Conidia: 0–septate, 7.5 per cent, 9.4 x 2.55μ
1–septate, 14.5 per cent, 13 x 3μ
2–septate, 3 per cent
3–septate, 48 per cent, 30.8 x 3.8μ
4–septate, 10 per cent, 38.8 x 4.03μ
5–septate, 17 per cent, 45.3 x 4.2μ

The organism was isolated only once from potato tubers, but it was isolated by the writer from a specimen of Rhizoctonia-infected tomato plant also, from Virginia, and by C. O. Dalrymple from ginseng garden soil in New York State. This seems to be a cosmopolitan species.

29. **Fusarium sanguineum** n. sp. (Fig. 25; Pl. III, figs. 7 and 8; Pl. VI, fig. 1)

Conidia typically strongly arcuate, gradually pointed toward apex, distinctly pedicellate, 3– to 5–septate — 3–septate conidia averaging 33.5 x 3.6 (24–37 x 3.4–3.8)μ and 5–septate averaging 45.2 x 4.1 (40–47 x 3.9–4.2)μ — single, in from small to medium-sized (up to 2 millimeters in diameter) sporodochia and in pseudopionnotes, the latter form of fructification dominant on most media, especially on agars; chlamydospores almost always present, intercalary in conidia and in mycelial threads, borne singly, in chains and in clusters; aërial mycelium seldom well developed and then from white to different shades of pink, on various agars mostly absent leaving exposed pseudopionnotes of ox-blood red color.

Hab. On rotted tubers of *Solanum tuberosum* in association with *F. lutulatum* var. *zonatum*, Ithaca, New York.

Latin description.—Conidiis typice valide arcuatis, gradatim in apicem acutis, distincte pedicellatis, 3–5–septatis — conidiis 3–septatis, 33.5 x 3.6 (24–37 x 3.4–3.8)μ, conidiis 5–septatis, 45.2 x 4.1 (40–47 x 3.9–4.2)μ — continuis, minutis vel mediocribus (usque ad 2 mm. diam.) sporodochiis et pseudopionnotibus, quo fructificationis modo in plurimis mediis frequentissimo imprimis in agaribus; chlamydosporis plerumque praesentibus, intercalaribus in conidiis et in hyphis, singulatim ortis, catenulatis vel uveosis; aerio mycelio raro plene maturo, sed, cum plene maturum, tum ex albo varie rubello tincto, in agaribus variis plerumque absente, pseudopionnotes "ox-blood red" (R) exponente.

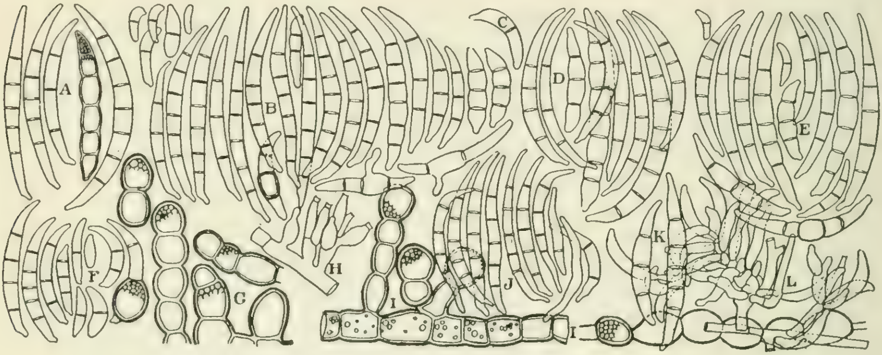

Fig. 25.—*Fusarium sanguineum.* A, *Pseudopionnotal conidia (one chlamydospore-like conidium) from 8-days-old culture on hard lima-bean agar with 2 per cent glucose;* B, *pseudopionnotal conidia from 14-dᴂys-old culture on hard lima-bean agar;* C, *typical conidium from 47-days-old culture on whole grains of wheat;* D, *pseudopionnotal conidia from 19-days-old culture on slightly acidified hard potato agar;* E, *pseudopionnotal conidia from 23-days-old culture on red raspberry cane plug;* F, *conidia,* G, *chlamydospores, from 76-days-old culture on potato tuber plug;* H, *conidiophores from 23-days-old culture on red raspberry cane plug;* I, *chlamydospores from 176-days-old culture on corn agar;* J, *sporodochial conidia from 41-days-old culture on rye straw;* K, *conidia from 176-days-old culture on corn meal;* L, *conidiophores from 47-days-old culture on whole grains of wheat*

Hab. In tuberibus putridis Solani tuberosi una cum F. lutulato var. zonato, Ithaca, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On red raspberry cane plug, culture twenty-three days old:

(1) Conidia from pseudopionnotes

Conidia: 1–septate, 7 per cent, 15 x 2.3 (10–23 x 2.2–2.5)μ
2–septate, 2.5 per cent
3–septate, 17 per cent, 32 x 3.4 (22–43 x 2.8–4.1)μ
4–septate, 7 per cent, 40 x 3.8 (35–45 x 3–4.3)μ
5–septate, 63.5 per cent, 47 x 4.2 (38–51 x 3.9–4.3)μ
6–septate, 3 per cent, 52–60 x 4.1–4.4μ (only a few measured)
7–septate, very few, 52–60 x 4.1–4.4μ (only a few measured, the thickest, swollen, 6.2μ in diameter)

(2) Chlamydospores
(a) Unicellular, in conidia, 6–12.5 x 6–8μ
(b) Unicellular, in mycelium, 8–17 x 7–14μ

On slightly acidified hard potato agar, culture nineteen days old; conidia from pseudopionnotes:

Conidia: 1–septate, 1 per cent
2–septate, 2 per cent
3–septate, 57 per cent, 37.5 x 3.6 (32–50 x 3–4)μ
4–septate, 17 per cent, 44 x 3.7 (38–60 x 3.5–4.4)μ
5–septate, 20 per cent, 45 x 4.1 (40–60 x 3.8–4.4)μ
$\left.\begin{array}{l}\text{6–septate}\\ \text{7–septate}\end{array}\right\}$ 3 per cent

On potato tuber plug, culture seventy-six days old; conidia from aërial mycelium:

Conidia: 0–septate, 5 per cent
1–septate, 15 per cent
2–septate, 10 per cent
3–septate, 55 per cent, 24 x 3.5 (17–35 x 3–4)μ
4–septate, 7 per cent
5–septate, 8 per cent, 40 x 4.1 (35–43 x 3.5–4.4)μ

On hard lima-bean agar with 2 per cent glucose, culture eight days old; conidia from pseudopionnotes:

Conidia: 3–septate, 3 per cent, 41 x 3.8 (35–44 x 3.5–4.1)μ
4–septate, 5 per cent
5–septate, 90 per cent, 47 x 4.2 (43–51 x 3.9–4.7)μ
6–septate, 2 per cent

On hard lima-bean agar, culture sixteen days old, most of the conidia swollen (only normal, not swollen, conidia, were measured):

Conidia: 1–septate, 9 per cent, 14.7 x 2.5 (12–19.5 x 2.3–3)μ
2–septate, very rare
3–septate, 37 per cent, 33 x 3.5 (18–41 x 3–4.1)μ
4–septate, 14 per cent
5–septate, 29 per cent, 47 x 3.9 (38–56 x 3.5–4.4)μ
6–septate, 7 per cent, 50 x 4 (43–58 x 3.9–4.4)μ
7–septate, 4 per cent, 50 x 4.1 (49–56 x 4–4.3)μ
8–septate, exceptional, 61 x 4.4μ (only one found and measured)

Average of the above measurements:

Conidia: 0–septate, 1 per cent
1–septate, 6 per cent
2–septate, 3 per cent
3–septate, 34 per cent, 33.5 x 3.6μ
4–septate, 10 per cent
5–septate, 42 per cent, 45.2 x 4.1μ
6– and 7–septate, 4 per cent, 50 x 4.05μ
8–septate, very rare, 61 x 4.4μ (only one measured)

30. *Fusarium sanguineum* var. **pallidium** n. var. (Figs. 1K and 26; Pl. III, figs. 5 and 6; Pl. VII, fig. 7)

Differs from *F. sanguineum* by better development of mycelium, by much slower rate of colony growth, by paler color of substratum and conidia (Pl. III, figs. 5 and 6), and by chlamydospores which are intercalary, as in *F. sanguineum*, but mostly in conidia and not in mycelium, and of much rarer occurrence than in *F. sanguineum*.

Hab. On rotted tubers of *Solanum tuberosum*, in association with *F. oxysporum*, South Dakota.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture three days old; conidia from pseudopionnotes:

Conidia: 2–septate, rare
3–septate, 53 per cent, 45 x 3.9 (38–56 x 3.5–4.1)μ
4–septate, 20 per cent, 40 x 4 (40–57 x 3.9–4.1)μ
5–septate, 20 per cent, 53 x 4.1 (47–60 x 3.9–4.3)μ
6– and 7–septate, 7 per cent, 55 x 4.3 (52–61 x 4.1–4.8)μ

On red raspberry cane plug, culture thirty-two days old; conidia from aërial mycelium:

Conidia: 1–septate, few
3–septate, 43 per cent, 30 x 3.8 (19–42 x 2.6–4.3)μ
4–septate, 36 per cent, 36 x 4 (31–42 x 3.5–4.3)μ
5–septate, 21 per cent, 39 x 4.1 (33–43 x 3.9–4.7)μ

On hard lima-bean agar with 2 per cent glucose, culture eight days old; conidia from pseudopionnotes:

Conidia: 3–septate, 60 per cent, 41 x 3.7 (35–48 x 3.5–4.1)μ
4–septate, 25 per cent, 51 x 3.8 (44–60 x 3.5–4.1)μ
5–septate, 15 per cent, 55 x 3.8 (52–56 x 3.5–4)μ

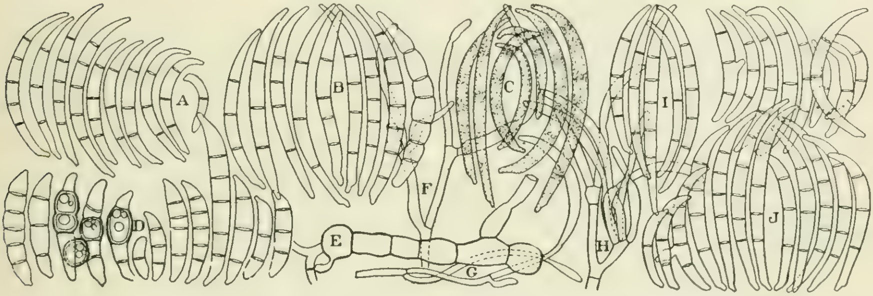

FIG. 26.— *Fusarium sanguineum var. pallidium.* A, *Pseudopionnotal conidia from 32-days-old culture on red raspberry cane plug;* B, *pseudopionnotal conidia from 3-days-old culture on slightly acidified hard potato agar;* C, *pseudopionnotal conidia with densely granulated content and indistinct septation from 8-days-old culture on hard lima-bean agar with 2 per cent glucose:* D, *conidia (normal, swollen, and with chlamydospores) from aërial mycelium from 35-days-old culture on whole steamed potato tubers;* E, *chlamydospore-like swellings in mycelium from 176-days-old culture on corn agar;* F, G, H, *conidiophores from 14-days-old culture on hard lima-bean agar;* I, *pseudopionnotal conidia from 35-days-old culture on whole steamed potato tubers;* J, *pseudopionnotal and aërial conidia from 14-days-old culture on hard lima-bean agar*

On whole steamed potato tuber, culture thirty-eight days old:

(1) From a tuft of conidiophores over dry surface of the tuber;[44] type of spores very much like that of *F. discolor* var. *triseptatum*

Conidia: 1–septate, 2 per cent
2–septate, 7 per cent, about 17 x 3.6μ
3–septate, 72 per cent, 24 x 4.5 (19–32 x 4–4.8)μ

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[44] This type is very different from the general type, and therefore was not taken into account for average size of conidia.

4–septate, 15 per cent, 26 x 4.7 (24–30 x 4.6–5.2)μ
5–septate, 4 per cent, 28 x 4.7 (26–32 x 4.2–5.2)μ, the largest 45.5 x 5.2μ

(2) From pionnotes over the cut surface of the tuber; spore type close to that of *F. arcuosporum*

Conidia: 3–septate, 68 per cent, 41 x 3.2 (29–48 x 2.9–3.5)μ
4–septate, 20 per cent, 44 x 3.4 (36–51 x 3.2–4)μ
5–septate, 12 per cent, 48 x 3.4 (40–57 x 3–3.7)μ

On hard lima-bean agar, culture fourteen days old; conidia from pseudopionnotes:

Conidia: 1–septate, 2 per cent, 21 x 2.8μ (only three measured)
2–septate, exceptional
3–septate, 83 per cent, 37 x 3.5 (33–42 [61 exceptional] x 3–3.9)μ
4–septate, 12 per cent
5–septate, 3 per cent, 47 x 3.8 (42–60 x 3.5–4)μ

On the same medium and of the same age as the above, but from a semi-dry minute sporodochium near the upper margin of the slant:

Conidia: 1–septate, 1 per cent
3–septate, 56 per cent, 43 x 3.8 (37–48 x 3.5–4.1)μ
4–septate, 29 per cent
5–septate, 14 per cent, 44 x 3.9 (40–49 x 3.5–4.2)μ

31. **Fusarium bullatum** n. sp. (Figs. $1A_1$ and 27)

Conidia typically somewhat less arcuate than the other species of the same section, less pointed toward the apex, and broader, usually distinctly pedicellate, mostly 5–septate, 42 x 4.3 (31–47 x 4.1–4.9)μ, from pale cream to salmon in color; chlamydospores intercalary in mycelium, mostly in chains and from small to large clusters; aërial mycelium nearly always well developed, of uniform medium height and density, nearly pure white in color; substratum on various agars from colorless to a tint of light buff.

Hab. On rotted tuber of *Solanum tuberosum*, together with *F. bullatum* var. *roseum* and with *F. oxysporum* var. *resupinatum*, in Iowa.

Latin description.—Conidiis typice paulo minus arcuatis quam ceteris ejusdem sectionis speciebus, minus in apicem acutis, latioribus, plerumque distincte pedicellatis, plerumque 5–septatis, 42 x 4.3 (31–47 x 4.1–4.9)μ, e pallide "cream color" (R) "salmon color" (R); chlamydosporis intercalaribus in mycelio, plerumque catenulatis, parvis vel magnis uveosis; aerio mycelio semper plene maturo, uniformi mediocri altitudine atque densitate, ferme pure albo; substrato in agaribus variis ex hyalino pallide luteolo tincto.

Hab. In tuberibus putridis Solani tuberosi una cum F. bullato var. roseo et cum F. oxysporo var. resupinato, Iowa, Amer. bor.

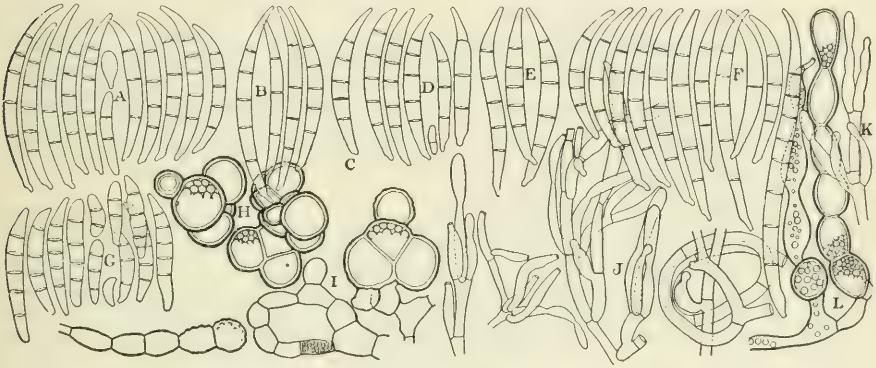

FIG. 27.—*Fusarium bullatum.* A, *Pseudopionnotal conidia from 10-days-old culture on hard lima-bean agar;* B, *pseudopionnotal conidia from 77-days-old culture on red raspberry cane plug;* C, *a typical conidium from 51-days-old culture on potato tuber plug;* D, *conidia from 76-days-old culture on potato tuber plug;* E, *conidia from 19-days-old culture on hard lima-bean agar;* F, *pseudopionnotal conidia from 7-days-old culture on hard potato agar;* G, *conidia,* H, *chlamydospores, from 42-days-old culture on rye straw;* I, *chlamydospores from 77-days-old culture on red raspberry cane plug;* J, *conidiophores from 7-days-old culture on hard potato agar;* K, *conidiophore from 10-days-old culture on hard lima-bean agar;* L, *chains of chlamydospores from 175-days-old culture on corn agar*

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture seven days old; conidia from pseudopionnotes:

Conidia: 3–septate, 16 per cent, 36 x 3.8 (29–50 x 3.5–4.9)μ
4–septate, 19 per cent, 43 x 4.1 (33–50 x 3.9–4.9)μ
5–septate, 65 per cent, 45 x 4.4 (37–63 x 4.1–4.9)μ
6– to 8–septate, rare, 65 x 4.9 (60–82 x 4.7–5.3)μ

On red raspberry cane plug, culture seventy-seven days old; conidia from aërial mycelium close to substratum:

Conidia: 3–septate, 25 per cent
4–septate, 20 per cent
5–septate, 55 per cent, 48 x 4.1 (42–53 x 3.5–4.4)μ

On potato tuber plug, culture seventy-six days old; conidia from aërial mycelium:

Conidia: 1–septate, 5 per cent
2–septate, 10 per cent
3–septate, 45 per cent, 31 x 3.7 (22–39 x 3.5–4.6)μ
4–septate, 20 per cent
5–septate, 20 per cent, 39 x 4.2 (35–45 x 4–4.4)μ

Same as preceding, culture eighty days old; conidia from aërial mycelium:

Conidia: 0–septate, 1 per cent
1–septate, 4 per cent
2–septate, 1 per cent
3–septate, 40 per cent, 30 x 3.7 (22–39 x 3.1–4.1)μ
4–septate, 20 per cent
5–septate, 34 per cent, 41 x 4.2 (35–49 x 3.5–4.7)μ
6– to 9–septate, rare (the largest, 9–septate, 56 x 5.7μ)

On hard lima-bean agar with 2 per cent glucose, culture nineteen days old; conidia from pseudopionnotes:

Conidia: 3–septate, 2 per cent, 35 x 3.8μ (only three measured)
4–septate, 1 per cent, 38 x 3.9μ (only four measured)
5–septate, 97 per cent, 47 x 4.4 (43–54 x 4–4.7)μ
6–septate, rare, 51 x 4.5μ (only three measured, 48–53 x 4.3–4.8μ)

On potato stem plug, culture one hundred and fourteen days old; conidia from aërial mycelium:

Conidia: 3–septate, 16 per cent
4–septate, 14 per cent
5–septate, 70 per cent, 41 x 4.35 (36–46 x 4–4.7)μ

On hard lima-bean agar, culture ten days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare
1–septate, 5 per cent
2–septate, rare
3–septate, 15 per cent
4–septate, 10 per cent
5–septate, 70 per cent, 43 x 4.1 (35–50 x 3.8–4.7)μ

In some cases 5–septate conidia were up to 100 per cent, in others conidia of lower septation were as high as 50 per cent. Of the smaller, 3–septate conidia were dominant.

On rye straw, culture fifty days old; conidia from aërial mycelium close to substratum:

Conidia: 3–septate, 10 per cent
4–septate, 5 per cent
5–septate, 85 per cent, 31.25 x 4.1 (27–36 x 3.9–4.6)μ

Average of the above measurements:

Conidia: 0–septate, rare
1–septate, 2 per cent
2–septate, 1.5 per cent
3–septate, 21 per cent, 33 x 3.75μ
4–septate, 15 per cent
5–septate, 60.5 per cent, 42 x 4.3μ
6– to 8–septate, very rare, 58 x 4.7μ

32. *Fusarium bullatum* var. **roseum** n. var. (Fig. 28; Pl. III, fig. 2)

Differs from *F. bullatum* mainly by its red substratum (on agar rich in glucose, see Plate III, figure 2) and by higher septation of conidia.

Hab. Same as that of *F. bullatum.*

This organism and *F. bullatum* are in general, especially in minute details of the character of the mycelium and in the chlamydospores, very much alike, and both were isolated from the same planting of diseased tissue of a rotted potato tuber and separated out on the first dilution. It is possible that they represent an example of a sudden and permanent variation of one Fusarium in pure culture.

IX. SECTION ELEGANS Wr., Phytopath. 3:28, fig. 1, E, F, S, T, U, V. 1913

Fusaria with ellipsoidal, 0– and 1–septate, microconidia; macroconidia typically 3–septate, often also 4– or 5–septate, more or less gradually pointed toward apex, pedicellate; chlamydospores intercalary and terminal in and on mycelial hyphæ, always present, and often also in and on conidia: color of conidia mostly pinkish buff; color of substratum, and of aërial mycelium when present (on potato agar rich in glucose and on rice), typically of various vinaceous hues, from light pinkish to dense purple.

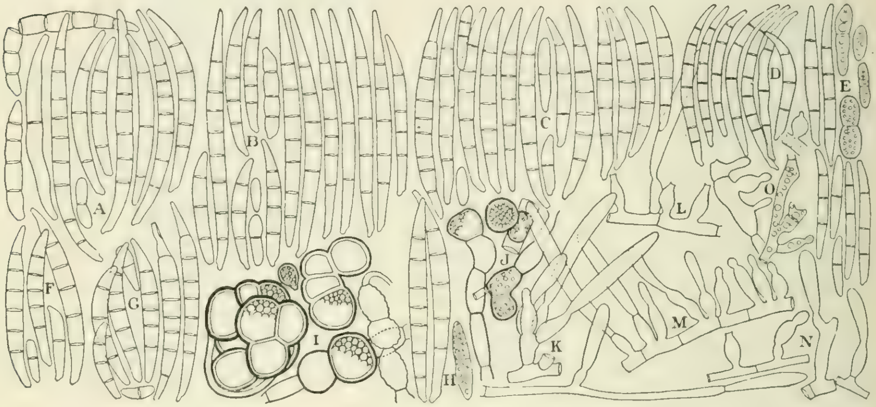

FIG. 28.—*Fusarium bullatum var. roseum.* A, *Pseudopionnotal conidia from 7-days-old culture on slightly acidified hard potato agar;* B, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar;* C, *pseudopionnotal conidia from 12-days-old culture on hard lima-bean agar;* D, *sporodochial conidia from 71-days-old culture on potato stem plug;* E, *pseudopionnotal conidia (many are swollen and with oil drops) from 35-days-old culture on corn meal;* F, *conidia from 37-days-old culture on hard oat gar;* G, *conidia from 67-days-old culture on red raspberry cane plug;* H, *pseudopionnotal conidia from 16-days-old culture on hard lima-bean agar (the two at the left typical for older growth, the one at the right for young growth);* I, *chlamydospores, intercalary in chains and in clusters, from 67-days-old culture on red raspberry cane plug;* J, *chlamydospores from 12-days-old culture on hard lima-bean agar;* K, L, M, *conidiophores from 7-days old culture on slightly acidified hard potato agar;* N, *conidiophores from 12-days-old culture on hard lima-bean agar;* O, *conidiophores from 35-days-old culture on corn meal*

33. *Fusarium orthoceras* Ap. et Wr. (Figs. 1, I_1 to K_1, and 29)

Appel, O., and Wollenweber, H. W. Arb. K. biol. Anst. Land- u. Forstw. 8:141–156; Pl. I, figs. 60 to 64; Pl. III, fig. 2. 1910. Wollenweber, H. W., Phytopath. 3:30, fig. 1s. 1913.

Microconidia always greatly in excess; macroconidia ranging from rare to several per cent of the total number of conidia, mostly nearly straight, sometimes slightly curved, typically 3-septate, 36 x 3.85 (25–40 x 3.2–4) μ; aërial mycelium usually well developed, from white to a tint of olive-buff; substratum, on potato agar rich in glucose, colorless at first, then from russet vinaceous to deep brownish vinaceous; no sporodochia; no pseudopionnotes; no sclerotia. Not the same as *F. oxysporum.*

Hab. Roots and tubers of Solanaceæ, also on various other hosts and in soil, in Europe and North America.

The organism was not isolated by the writer. The original culture was obtained from the Centralbureau der Association Internationale des Botanistes, Amsterdam, Holland, and was studied in conjunction with the other Fusaria. The above description is in the main the same as that given by Wollenweber.

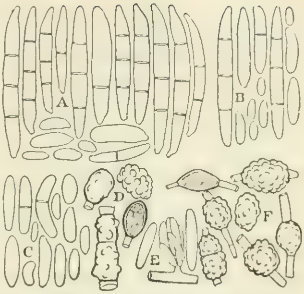

Fig. 29.— *Fusarium orthoceras.* A, *Conidia from 18-days-old culture on slightly acidified hard potato agar;* B, *conidia from 26-days-old culture on potato tuber plug;* C, *conidia from 15-days-old culture on hard lima-bean agar with 2 per cent glucose;* D, *chlamydospores from 18-days-old culture on slightly acidified hard potato agar;* E, *conidia, some in a ball, with conidiophore of common type, from 30-days-old culture on corn agar;* F, *chlamydospores from 53-days-old culture on potato tuber plug*

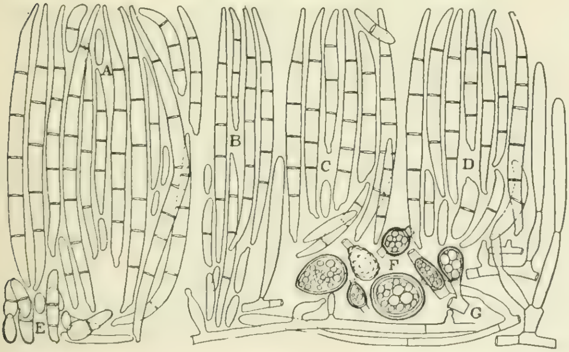

Fig. 30.— *Fusarium angustum.* A, *Pseudopionnotal conidia from 4-days-old culture on slightly acidified hard potato agar;* B, *conidiophores from surface of the exposed substratum from 25-days-old culture on corn agar;* C, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar with 2 per cent glucose;* D, *pseudopionnotal conidia from 8-days-old culture on hard lima-bean agar;* E, *microconida from aërial mycelium;* F, *chlamydospores from 74-days-old red raspberry cane plug;* G, *conidiophores from 9-days-old culture on hard lima-bean agar with 2 per cent glucose*

34. **Fusarium angustum** n. sp. (Figs. 1, G_1 and H_1, and 30)

Conidia gradually pointed toward apex, from slightly curved to nearly straight or anguiform, usually distinctly pedicellate, mostly 3–septate, 45.64 x 3.52 (42–49 x 3.3–3.6)μ, often 0– to 5–septate, sometimes 6– to 8–septate; on various agars usually producing thin pseudopionnotes, otherwise very similar to *F. oxysporum.*

Hab. In discolored fibrovascular bundles of tubers of *Solanum tuberosum,* Ithaca, New York.

Latin description.—Conidiis gradatim in apicem acutis, paulum curvatis vel prope rectis anguiformibusque, plerumque distincte pedicellatis, plerumque 3 septatis, 45.64 x 3.52 (42–49 x 3.3–3.6)μ, saepe 0–5–septatis, interdum 6–8–septatis; in variis agaribus tenues pseudopionnotes plerumque exhibentibus, aliter simillimis F. oxysporo.

Hab. In tuberum fasciculis decoloratis fibro-vascularibusque Solani tuberosi, Ithaca, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture four days old; conidia from pseudopionnotes:

Conidia: 0–septate, 6 per cent, 12 x 2.5 (8–18 x 1.8–3.5)μ
1–septate, 7 per cent, 32 x 2.9 (19–52 x 2.5–4.2)μ
2–septate, 2 per cent, 32 x 2.7 (30–39 x 2.5–3)μ
3–septate, 44 per cent, 42.2 x 3.3 (31–69 x 2.5–4.4)μ
4–septate, 13 per cent, 60.4 x 3.8 (47–70 x 3.5–4.4)μ
5–septate, 27 per cent, 63 x 3.9 (43–81 x 3.5–4.1)μ
6– to 8–septate, 1 per cent, 90 x 4.1 (80–102 x 4–4.4)μ

On red raspberry cane plug, culture seventy-four days old:

Conidia: 0–septate, 90 per cent
1–septate, 7 per cent
3–septate, 3 per cent, 39.4 x 3.81 (30–50 x 3–4.8)μ

On hard lima-bean agar with 2 per cent glucose, culture nine days old; conidia from pseudopionnotes:

Conidia: 0–septate, 54 per cent, 10 x 2.8 (5–15 x 2.7–3.1)μ
1–septate, 7 per cent, 18 x 2.9 (15–22 x 2.7–3.1)μ
2–septate, 3 per cent
3–septate, 20 per cent, 43 x 3.6 (29–53 x 3–4.1)μ
4–septate, 8 per cent
5–septate, 8 per cent, 59 x 4.5 (52–70 x 3.9–4.8)μ
6–septate, few, 70 x 4.7μ (only one measured)

On hard lima-bean agar, culture eight days old; conidia from pseudopionnotes (measurements made in April):

Conidia: 0–septate, 39 per cent, 11 x 2.6 (8.7–14 x 2.1–3.2)μ
1–septate, 17 per cent, 18 x 3.2 (14–23 x 2.9–3.9)μ
2–septate, 3 per cent, 24 x 3.3μ (only four measured)
3–septate, 32 per cent, 49 x 3.6 (42–55 x 3.5–4.8)μ
4–septate, 2 per cent, 53 x 4μ (only four measured)
5–septate, 6 per cent, 58 x 4.3 (52–65 x 3.5–4.8)μ
6–septate, 1 per cent, 73 x 4.7μ (only one measured)

On same media, culture nine days old (measurements made in May):

Conidia: 0–septate, 11 per cent, 10 x 2.5 (5–15 x 1.6–3.3)μ
1–septate, 9 per cent, 17 x 3.1 (12–24 x 2.6–4)μ
2–septate, 1 per cent
3–septate, 43 per cent, 49 x 3.6 (31-54 x 2.7–4.1)μ
4–septate, 13 per cent
5–septate, 23 per cent, 50 x 4 (54–71 x 3.5–4.7)μ

Average of the above measurements:

Conidia: 0–septate, 27.5 per cent, 11 x 2.6μ
1–septate, 10 per cent, 21 x 3μ
2–septate, 2 per cent, 28 x 3μ
3–septate, 35 per cent, 45.64 x 3.52μ
4–septate, 9 per cent, 56.7 x 3.9μ
5–septate, 16 per cent, 60 x 4.2μ
6–septate, 0.5 per cent, 78 x 4.5μ
8–septate, very rare, 78 x 4.5μ

35. *Fusarium redolens* Wr.[45] var. **Solani** n. var. (Figs. 1P$_1$ and 31; Pl. II, figs. 3 and 4; Pl. V, fig. 2)

Macroconidia typically more or less gradually attenuate, sometimes suddenly constricted at the apex, pedicellate, typically broader toward and more curved near apex, 3-septate, 36.4 x 4.86 (31–41 x 4.3–5)μ, often also 4- and 5-septate, usually in numerous from small to medium (up to $2\frac{1}{2}$ millimeters, commonly $\frac{1}{2}$ millimeter, in diameter) sporodochia, sometimes, especially on different agars and in an early stage of culture growth, in pseudopionnotes; from nearly white in color when in small powdery masses, to a bright orange color when in comparatively large sporodochia on nearly dry potato stems; mostly, however, from a light pinkish cinnamon to a pinkish buff; aërial mycelium sometimes medium well developed but usually very scant, short, from white to somewhat grayish or brownish gray in color (Pl. II, fig. 3); color of substratum, on agar rich in glucose, as shown on Plate II, figure 4, which is different from all the other Fusaria of the section Elegans.

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[45] *Fusarium redolens* Wr. is described by its author (see Wollenweber, Phytopath. **3**: 29–30, fig. 1 E, 1913) as follows: "Differs from all the above-mentioned species [*F. oxysporum*, *F. tracheiphilum*, *F. vasinfectum*, *F. vasinfectum* var. *inodoratum*, *F. lycopersici*, and *F. niveum*] in the large size of its triseptate conidia, 30–40 x 4.5–5.5μ, and in the color of the brownish white conidial masses. A lilac odor is produced on rice and milk. No blue sclerotia. Vascular parasite, cause of wilt and foot disease of *Pisum sativum*. Distribution unknown."

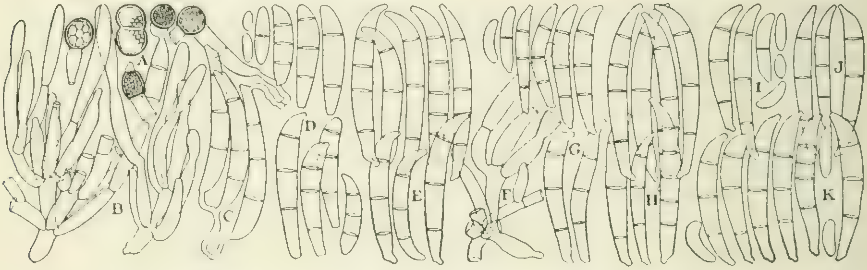

FIG. 31.—*Fusarium redolens var. Solani.* A, *Chlamydospores produced by conidia and mycelium from 120-days-old culture on potato stem plug;* B, *conidiophores from 14-days-old culture on slightly acidified hard potato agar;* C, *anastomosed conidia from 13-days-old culture on hard lima-bean agar;* D, *sporodochial conidia from 35-days-old culture on potato stem plug;* E, *pseudopionnotal conidia,* F, *conidiophores, from 14-days-old culture on slightly acidified hard potato agar;* G, *sporodochial conidia from 63-days-old culture on whole grain of rye;* H, *normal pseudopionnotal conidia from 13-days-old culture on hard lima-bean agar;* I, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar with 2 per cent glucose (the longest spore is typical);* J, *pseudopionnotal conidia from 120-days-old culture on potato stem plug;* K, *sporodochial conidia from 48-days-old culture on red raspberry cane plug*

Hab. On rotted tubers of *Solanum tuberosum*, Atlanta, New York.

Differs from *F. redolens* Wr. by somewhat narrower conidia and by color of substratum,[46] especially on potato agar rich in glucose.

Measurements of spores on different media are as follows:

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[46] Wollenweber does not directly give color of substratum of his *F. redolens*, but judging from his definite negative statement in regard to *F. conglutinans* Wr. it appears evident that *F. redolens* does not differ noticeably in color from *F. oxysporum* and the majority of other species of the section Elegans.

On slightly acidified hard potato agar, culture fourteen days old; conidia from pseudopionnotes:

Conidia: 2–septate, 0.5 per cent, 26 x 4.7μ
3–septate, 93.5 per cent, 36 x 5 (26–49 x 4.3–5.8)μ
4–septate, 6 per cent, 41.6 x 5.3 (38–46 x 4.9–6.1)μ
5– and 6–septate, rare, 43–53 x 5–6μ

On red raspberry cane plug, culture forty-eight days old; conidia from a small sporodochium:

Conidia: 0–septate, 2 per cent
1–septate, 1 per cent
3–septate, 97 per cent, 35.6 x 5 (28–45 x 4–5.9)μ

On hard lima-bean agar, culture nine days old; conidia from pseudopionnotes:

Conidia: 0–septate, 17 per cent, 10 x 3.6 (6–16 x 3–4.3)μ
1–septate, 10 per cent, 20 x 3.9 (14–28 x 3.5–4.7)μ
2–septate, 3 per cent
3–septate, 70 per cent, 34 x 4.9 (28–43 x 4.3–5.3)μ
4–septate, rare

On potato tuber plug, culture ninety-nine days old; conidia from a medium small sporodochium:

Conidia: 2–septate, rare, 27 x 4.8μ
3–septate, 100 per cent, 33.6 x 4.9 (29–39 x 4.6–5.7)μ

On potato stem plug, culture one hundred and thirteen days old:
(1) Chlamydospores, mostly unicellular
Chlamydospores: 0–septate, 6.5 x 6.1 (4.7–11 x 5.2–7.5)μ
(2) Conidia from a sporodochium
Conidia: 0– and 1–septate, very rare
3–septate, 100 per cent, 34.5 x 4.5 (24–39 x 3.9–5.1)μ
4–septate, very rare, about the same size as 3–septate

On whole steamed potato tuber, culture forty-nine days old; conidia from a medium small sporodochium:

Conidia: 0– and 1–septate, rare
3–septate, 100 per cent, 31 x 4.3 (29–36 x 4.1–4.8)μ

On rye grain, culture sixty-three days old; conidia from a small sporodochium:

(1) Conidia: 0–septate, 5 per cent
1–septate, 3 per cent
2–septate, 1 per cent
3–septate, 91 per cent, 34.6 x 4.6 (28–41 x 3.8–5.2)μ
4–septate, rare
5–septate, very rare

(2) Chlamydospores in mycelium, terminal, about the same size as those on potato stem plug

On corn agar, culture one hundred and seventy-three days old, chlamydospores intercalary and terminal:

Chlamydospores: 0–septate (in conidia), 9.4 x 8.3 (7–11 x 5.2–9)μ
1–septate (in mycelium), 15 x 9 (11–20 x 7–11)μ

On hard lima-bean agar with 2 per cent glucose, culture one hundred and eighteen days old:

(1) Chlamydospores not numerous; in conidia, 0–septate, 8.7 x 8.5μ; in mycelium, 0– and 1–septate, and also in small clusters of three

(2) Sporodochial conidia

Conidia: 1–septate, rare
3–septate, 94 per cent, 37 x 5 (28–44 x 4.6–5.9)μ
4–septate, 6 per cent, 40 x 5.05 (37–44 x 4.7–5.9)μ
5–septate, rare, about the same size as 4–septate

On hard lima-bean agar, culture five days old; chlamydospores (in conidia) not observed; conidia from pseudopionnotes:

Conidia: 0–septate, 10 per cent, 11 x 3.6μ
1–septate, 3 per cent, 22 x 4μ
2–septate, 1 per cent
3–septate, 86 per cent, 41 x 4.9 (33–53 x 4.6–5.2)μ
4–septate, rare
5–septate, very rare

On the same medium as above, culture thirteen days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare
3–septate, 97 per cent, 41 x 4.9 (34–49 x 4.2–5.2)μ
4–septate, 3 per cent, 43 x 5 (36–48 x 4.7–5.2)μ
5–septate, rare, 45 x 5.1 (43–47 x 4.8–5.2)μ

Average of the above measurements:
Conidia: 0–septate, 3.5 per cent, 10.5 x 3.6μ
1–septate, 1.5 per cent, 21 x 3.95μ
2–septate, 0.5 per cent, 26.5 x 4.75μ
3–septate, 93 per cent, 36.4 x 4.86μ
4–septate, 1.5 per cent, 41.5 x 5.12μ
5–septate, rare, 44.3 x 5.2μ
6–septate, very rare

36. **Fusarium lutulatum** n. sp. (Figs. 1F$_1$ and 32; Pl. II, figs. 5 and 6; Pl. V, fig. 3)

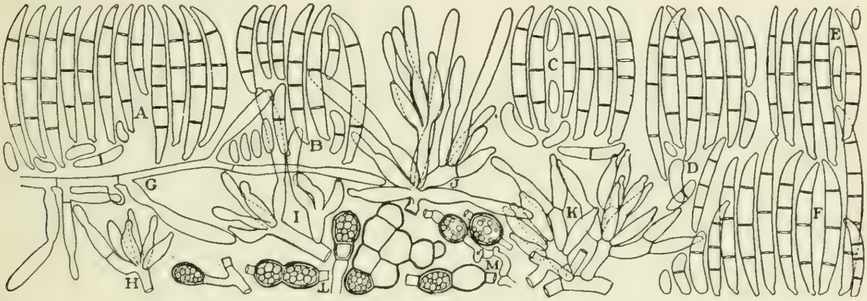

Fig. 32.—*Fusarium lutulatum.* A, *Conidia from plectenchymic sporodochium from 60-days-old culture on red raspberry cane plug;* B, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar with 2 per cent glucose;* C, *pseudopionnotal conidia from 5-days-old culture on hard lima-bean agar;* D, *sporodochial conidia from 63-days-old culture on whole grain of rye;* E, *sporodochial conidia from 49-days-old culture on whole steamed potato tuber;* F, *pseudopionnotal conidia from 23-days-old culture on slightly acidified hard potato agar;* G, H, I, J, *conidiophores from 5-days-old culture on hard lima-bean agar;* K, *conidiophores from 23-days-old culture on slightly acidified hard potato agar;* L, *terminal and intercalary chlamydospores from 63-days-old culture on whole grain of rye;* M, *chlamydospores from 25-days-old culture on corn agar*

Macroconidia gradually attenuate toward the apex, usually distinctly pedicellate and uniformly curved throughout, without stronger curvature near apex, typically 3–septate, 34 x 4 (28–38 x 3.7–4.5)μ, also 2– to 5–septate; from small to medium sporodochia (up to 2 millimeters in diameter), often converging into pseudopionnotes; aërial mycelium, when present, short, (mostly from 1 to 2 millimeters high), white, often, especially on agars in plate cultures, absent; color of conidia from nearly white (on aërial mycelium in the form of coarse powder) to dark vinaceous purple; typical variation of spore color shown in Plate II, figures 5 and 6; sometimes on potato stem plug, from one to a few large sporodochia (2 millimeters in diameter) of a bright orange color produced; substratum from colorless to that of the conidial masses; small bluish black sclerotia (½ millimeter in diameter) sometimes produced, and then in great numbers all over the substratum (on potato tuber plug); zonation of colony very faint or none on neutral agars in plate cultures.

Hab. On soft and dry rotted tubers of *Solanum tuberosum*, Atlanta, New York.

Latin description.—Macroconidiis gradatim in apicem attenuatis, plerumque distincte pedicellatis et aequabiliter curvatis, non ad apicem curvatioribus, typice 3–septatis, 34 x 4.23 (28–38 x 3.7–4.5)μ, vel etiam 2–5–septatis; sporodochiis minutis vel mediocribus (usque ad 2 mm. diam.), saepe in pseudopionnotes vergentibus; aerio mycelio saepe — praecique in agaribus in culturis in patellis factis — absente; conidiis ex albido (in aerio mycelio similibus magnis granis pulveris) "dark vinaceous purple" (R); vide typicam spororum coloris variationem, Tab. II, figg. 5, 6; interdum in tuberibus Solani tuberosi, vel unis vel paucis sporodochiis (2 mm. diam.) nitide "orange" (R); substrato hyalino vel eodem colore quo conidiorum moles; interdum sclerotiis minutis livido-atris (½ mm. diam.), tum demum eorundem magnis numeris passim in substrato in tuberibus Solani tuberosi; zonatione coloniae vel maxime indistincta vel nulla in agaribus neutralibus in culturis in patellis factis.

Hab. In mollibus vel aridis putridis tuberibus Solani tuberosi, Atlanta, New York, Amer. bor.

Measurements of spores on different media are as follows:

On slightly acidified hard potato agar, culture twenty-three days old; conidia from pseudopionnotes:

Conidia: 0–septate, 4.5 per cent, 9 x 2.7 (6–14 x 2.5–3)μ
1–septate, 1 per cent, 18 x 3.2 (13–22 x 2.6–4)μ
2–septate, 0.5 per cent, 19.5 x 3.2 (17–23 x 2.7–3.5)μ
3–septate, 89 per cent, 35.2 x 4.5 (23–38 x 3.5–4.7)μ
4–septate, 4.8 per cent, 37.8 x 4.6 (31–40 x 4.3–4.7)μ
5–septate, 0.2 per cent, 45 x 4.7 (38–50 x 4.7)μ

On red raspberry cane plug, culture sixty days old; conidia from a medium-sized sporodochium:

Conidia: 0–septate, 25 per cent
1–septate, 5 per cent
2–septate, 1 per cent
3–septate, 65 per cent, 38 x 4.2 (27–44 x 3.5–4.7)μ
4–septate, 4 per cent, 42 x 4.4 (36–49 x 3.7–4.7)μ
5–septate, rare, about the size of 4–septate conidia

On hard lima-bean agar, culture nine days old; conidia from aërial mycelium:

Conidia: 0–septate, 57 per cent, 8.5 x 2.8 (7–12 x 2.6–3.5)μ
1–septate, 16 per cent, 14.5 x 3.25 (11–21 x 2.9–3.8)μ
2–septate, 3.5 per cent
3–septate, 23 per cent, 28 x 3.9 (23–37 x 3.5–4.4)μ
4–septate, 0.5 per cent, 35 x 3.9 (30–37 x 3.5–4.4)μ

On same media, culture twenty-two days old; conidia from small sporodochia:

Conidia: 0–septate, 3 per cent
1–septate, 1 per cent
2–septate, rare
3–septate, 84 per cent, 36 x 4 (29–40 x 3.9–4.4)μ
4–septate, 10 per cent, 39 x 4.3 (33–42 x 3.9–4.7)μ
5–septate, 2 per cent, 41 x 4.2μ (only a few measured)

On hard potato agar, culture thirty-one days old:

(1) Conidia from pseudopionnotes

Conidia: 0–septate, 26 per cent, 7.4 x 2.7 (5.2–12 x 2.6–3.5)μ
1–septate, 4 per cent, 13 x 3.3μ (only three measured)
2–septate, rare, 20 x 3.7μ (only three measured)
3–septate, 54 per cent, 35 x 4.2 (20–41 x 3.4–4.8)μ
4–septate, 12 per cent, 40 x 4.4 (35–44 x 4–4.8)μ
5–septate, 4 per cent, 43 x 4.8 (41–46 x 4–4.8)μ

(2) Chlamydospores (abundant)
  (a) Terminal, 0–septate, on mycelium, 8 x 6.6 (7–9 x 6–7)μ
  (b) Intercalary, in mycelium
    0–septate, 8.2 x 6.2 (6–9 x 4.5–8)μ
    1–septate, 11 x 5.7μ (only a few measured)
  (c) In conidia, 0–septate, 6.5 x 5.9μ

On potato tuber plug, culture ninety-nine days old:
(1) Conidia from a sporodochium
Conidia: 0–septate, 5 per cent, 6.2 x 2.4 (4.5–8 x 2–2.9)μ
1–septate, 1 per cent, 13 x 2.7μ (only a few measured)
2–septate, rare, 20 x 3.3μ (only a few measured)
3–septate, 92 per cent, 32 x 3.8 (20–38 x 3–4.2)μ
4–septate, rare, 37 x 4.1μ (only one measured)
5–septate, very rare, about the size of 4–septate
(2) Chlamydospores, numerous, mostly in conidia
0–septate, 6.8 x 5.1 (5.2–7.2 x 4.6–5.3)μ
1–septate, 9.6 x 5.1 (7.8–12.3 x 4.1–6)μ, the largest 8.8 x 6.1μ

On potato stem plug, culture one hundred and twelve days old:
(1) Conidia from a sporodochium
Conidia: 0–septate, 1 per cent
1–septate, rare
3–septate, 79 per cent, 36.4 x 4.1 (33–39 x 3.9–4.7)μ
4–septate, 13 per cent, 40 x 4.2 (36–42 x 3.9–4.4)μ
5–septate, 7 per cent, 40 x 4.3 (35–45 x 4–4.7)μ
6–septate, very rare, same size as 5–septate
(2) Chlamydospores, in conidia common, mostly 0–septate, 6.3 x 5.2 (5.1–8 x 4.3–6)μ

On whole steamed potato tuber, culture forty-nine days old; conidia from a sporodochium:
Conidia: 0–septate, 4 per cent
3–septate, 86 per cent, 36 x 3.7 (22–44 x 2.9–4.1)μ
4–septate, 8 per cent, 38 x 4.1 (35–43 x 3.9–4.2)μ
5–septate, 2 per cent, 40 x 4.1 (37–44 x 4.1)μ

On rye grain, culture sixty-three days old; conidia from a small sporodochium:
Conidia: 0–septate, 35 per cent
1–septate, 12 per cent
2–septate, 1 per cent
3–septate, 45 per cent, 34 x 4 (24–44 x 3.5–4.4)μ
4–septate, 5 per cent, 41 x 4.2 (36–47 x 4–4.7)μ
5–septate, 2 per cent, 45 x 4.3 (44–51 x 4–4.7)μ

On corn agar, culture one hundred and seventy-two days old; most of the conidia with chlamydospores:

(1) Pseudopionnotal conidia (only those without chlamydospores were measured)
Conidia: 3–septate, 32 x 4μ
4–septate, 35 x 4.1μ
5–septate, 37 x 4.2μ
(2) Chlamydospores in conidia very numerous, 0–septate predominant, 7.3 x 6.8 (6–10 x 5–9)μ

On hard lima-bean agar with 2 per cent glucose, culture one hundred and eighteen days old:

(1) Chlamydospores in conidia very numerous, 0–septate predominant, 7.2 x 5.7 (6–8 x 5.2–6)μ
(2) Sporodochial conidia
Conidia: 3–septate, 99 per cent, 33 x 4.2 (29–39 x 3.8–4.7)μ
4–septate, 1 per cent, 36 x 4.3 μ (only three measured)

On hard lima-bean agar, culture five days old; conidia from aërial mycelium (chlamydospores in conidia not observed):

Conidia: 0–septate, 46 per cent, about 11 x 2.6μ
1–septate, 4 per cent, about 16 x 3.1μ
2–septate, 1.5 per cent
3–septate, 48 per cent, 32 x 4 (23–41 x 3.5–4.3)μ
4–septate, 0.5 per cent, 35 x 4.1μ (only two measured)

On same medium as above, culture thirteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 25 per cent
1–septate, 6 per cent
2–septate, 1 per cent
3–septate, 63 per cent, 35 x 4.2 (30–40 x 3.9–4.7)μ
4–septate, 5 per cent, 37.8 x 4.4 (33–41 x 4–4.7)μ

Average of the above measurements:

Conidia: 0–septate, 20 per cent 8.4 x 2.6μ
1–septate, 1.5 per cent, 15 x 3.2μ
2–septate, 3.5 per cent, 20 x 3.4μ
3–septate, 69 per cent, 33.9 x 4.07μ
4–septate, 5 per cent, 39.4 x 4.23μ
5–septate, 1 per cent, 41.1 x 4.32μ

37. *Fusarium lutulatum* var. **zonatum** n. var. (Pl. I, figs. 9 and 10; Pl. V, fig. 4)

Differs from *F. lutulatum* by slightly shorter and broader microconidia; usually by the absence of chlamydospores in conidia;[47] by conidia somewhat less pointed than those in *F. lutulatum*; by commoner production of aërial mycelium; and by more or less distinct zonation of colony growth on neutral agars. Spore color begins to develop earlier but usually does not reach the density of that of *F. lutulatum*. The organism does not produce rot of potato tubers, while *F. lutulatum*, at least in most of the inoculations made, is capable of causing such rot. No sclerotia observed.

Hab. On rotted tubers of *Solanum tuberosum* together with *F. oxysporum* var. *resupinatum* and *F. sanguineum*, at Ithaca, New York.

The size of the conidia is in many instances almost identical with that in *F. lutulatum*, and in general there is scarcely any very sharp distinction between the two organisms; but there are at least slight differences in many of the important characters, so that in general there can hardly be any serious doubt that these are two distinct, though closely related, organisms.

Average of the measurements of conidia on the same media, of the same age, and the same in other ways, as those of *F. lutulatum*, is as follows:

Conidia: 0–septate, 23 per cent, 9 x 2.8μ
1–septate, 5 per cent, 16 x 3.1μ
2–septate, 2 per cent, 22 x 3.5μ
3–septate, 69 per cent, 32.77 x 4.16μ
4–septate, 1 per cent, 39.4 x 4.4μ
5–septate, very rare, 39.3 x 4.6μ

38. **Fusarium sclerotioides** n. sp. (Figs. 10$_1$ and 33; Pl. I, figs. 11 and 12; Pl. V, fig. 1)

Microconidia gradually attenuate toward and more or less pointed at the apex, pedicellate, generally somewhat more distinctly curved near apex, and broader in the middle or in the upper third of their length, typically 3–septate, 34.7 x 4.4 (30–39.5 x 4.1–4.6)μ, also 2– to 5–septate, 4– and 5–septate being of more or less common occurrence; chlamydospores observed only in mycelium (intercalary and terminal), and not very common nor numerous when compared with all the other species of section Elegans, usually only unicellular; aërial mycelium on hard agars invariably well developed, of medium height (from 2 to 4 millimeters) and density, very frequently forming macroscopically observable knots at the hyphal tips, finally, if the conditions are right, resulting in production of numerous small sporodochia; large (up to 12 millimeters in diameter), bluish black, shiny, more or less wrinkled, sclerotia are frequently produced on potato tuber plugs; the sclerotia in some cases overgrown with aërial mycelium, and then not so conspicuous; plectenchymic bodies (from 1 to 3 or more millimeters in diameter) wartlike in appearance, white or pale flesh in color, often produced in considerable number and in some cases finally bearing masses of septate conidia; conidia, however, rarely produced on the sclerotia; color of the conidial mass somewhat variable, but usually of a tint of pinkish buff; color of substratum varying from nearly colorless when young to cinnamon red, deep vinaceous, and dark vinaceous purple. (For typical color when mature see Plate I, figure 12.)

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[47] These were observed in number only once, and then when the culture was very old and much contaminated with bacteria. It was observed generally that a bacterial contamination greatly stimulates production of chlamydospores.

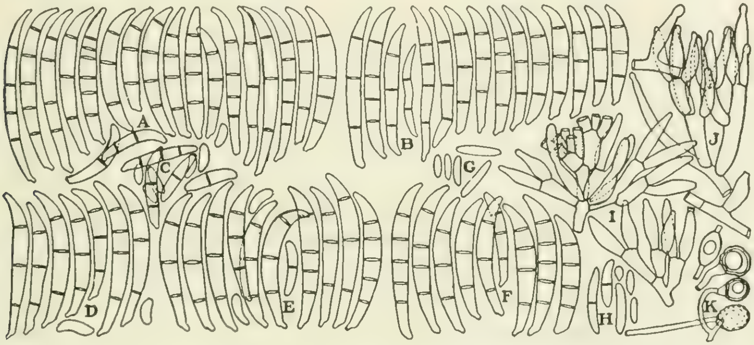

Fig. 33.— *Fusarium sclerotioides.* A, *Conidia from plectenchymic sporodochium from 14-days-old culture on potato tuber plug;* B, *conidia from plectenchymic sporodochium from 58-days-old culture on red raspberry cane plug;* C, *conidia from 24-days-old culture on slightly acidified hard potato agar;* D, *sporodochial conidia from 6-days-old culture,* E, *sporodochial conidia from 12-days-old culture,* F, *sporodochial conidia from 51-days-old culture, on hard lima-bean agar;* G, *microconidia from 13-days-old culture on hard lima-bean agar with 2 per cent glucose;* H, *microconidia from aërial mycelium,* I, *sporodochial conidiophores, from 58-days-old culture on red raspberry cane plug;* J, *conidiophore from 6-days-old culture on hard lima-bean agar;* K, *chlamydospores from 58-days-old culture on red raspberry cane plug*

Hab. On rotted tubers and in discolored fibrovascular bundles of tubers of *Solanum tuberosum* at Atlanta and Ithaca, New York, and in Louisiana.

Cultures of this organism on all media may for a long time produce microconidia almost exclusively; but if mature macroconidia from occasionally produced sporodochia are planted, macroconidia may be produced again in new cultures in great abundance and on almost any medium.

Latin description.—Microconidiis gradatim in apicem attenuatis, apice acutis, pedicellatis plerumque aliquatenus distinctius ad apicem curvatis, medio latioribus vel superiore tertio longitudinis, typice 3–septatis, 34.7 x 4.4 (30–39.5 x 4.1–4.6)μ, vel etiam 2–5–septatis, plus minusve saepe 4–5–septatis; chlamydosporis tantum in mycelio (intercalaribus et terminalibus) sed nec maxime frequentibus nec multis prae ceteris sectionis Elegantis speciebus, plerumque tantum unicellularibus; aerio mycelio in duris agaribus semper plene maturo, mediocriter alto (2–4 mm.) densoque, saepissime nodos nudo oculo conspicuos formante in hyphalibus apicibus, demum — condicionibus faventibus — multa minuta sporodochia ferente; saepe sclerotiis magnis (usque ad 12 mm. diam.) livido–atris, lucidis, plus minusve rugosis, in tuberibus Solani tuberosi; sclerotiis — si aerio mycelio obsita — minus conspicuis; saepe multis plectenchymicis corporibus (1–3 mm. diam., vel majoribus), verrucoideis, albis vel pallide carneis, vel moles septatorum conidiorum vel nullas tandem ferentibus; conidiis autem in sclerotiis raro ortis; colore conidiorum molis aliquatenus variante, sed plerumque "pinkish buff" (R) tincto; colore substrati juvenis ferme hyalino dein "cinnamon rufous" (R), "deep vinaceous" (R), "dark vinaceous purple" (R). (Vide typicam colorem maturi substrati Tab. I, fig. 12.)

Hab. In putridis tuberibus et in decoloratis fibro-vascularibus fasciculis tuberum Solani tuberosi, Atlanta et Ithaca, New York, et Louisiana, Amer. bor.

Measurements of conidia on different media are as follows:

On red raspberry cane plug, culture fifty-eight days old; conidia from a sporodochium:

Conidia: 1–septate, 1 per cent
2–septate, 1.5 per cent
3–septate, 87 per cent, 30 x 4.4 (21–40 x 3–5.1)μ
4–septate, 9 per cent, 37 x 4.5 (33–41 x 4.1–5.1)μ
5–septate, 1.5 per cent, 40 x 4.6 (35–45 x 4.3–5.1)μ

On hard lima-bean agar with 2 per cent glucose, culture thirteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 90 per cent, 9.5 x 2.25 (7–15.5 x 1.75–2.4)μ
1–septate, 10 per cent, 13.4 x 2.5 (12–18 x 2.3–3)μ

On corn meal agar, culture one hundred and seventy-three days old:

Chlamydospores: 0–septate, in mycelium, 8.5 x 8.2 (7–11.5 x 5–10.5)μ

On hard lima-bean agar, culture six days old; conidia from a sporodochium:

Conidia: 0–septate, rare
1–septate, very rare
3–septate, up to 100 per cent, 37 x 4.3 (31–41 x 3.9–4.4)μ
4–septate, rare, 37 x 4.4μ (only one measured)

On same medium as above, culture fifty-one days old; conidia from a medium-sized sporodochium:

Conidia: 0–septate, 1 per cent
1–septate, 1.5 per cent
3–septate, 73 per cent, 33 x 4.5 (23–39 x 3.5–4.8)μ
4–septate, 23 per cent, 37 x 4.5 (31–42 x 4–4.8)μ
5–septate, 1.5 per cent, 38 x 4.6 (31–42 x 4.2–4.9)μ

On same medium as above, culture twelve days old; conidia from pseudopionnotes:

Conidia: 0–septate, 6 per cent
1–septate, 3 per cent
2–septate, exceptional
3–septate, 74 per cent, 35 x 4.6 [(22–) 33–39 x (3.5–) 4.1–4.8]μ
4–septate, 16 per cent, 36 x 4.7 (33–42 x 4.4–4.8)μ
5–septate, 1 per cent, 37 x 4.7 (35–40 x 4.6–4.8)μ

On potato tuber plug, culture fourteen days old; conidia from a medium-sized sporodochium:

Conidia: 0–septate, 8 per cent
1–septate, 3 per cent
2–septate, rare
3–septate, 46 per cent, 35 x 4.1 (29–43 x 3–4.7)μ
4–septate, 29 per cent, 40 x 4.2 (31–47 x 3.5–4.7)μ
5–septate, 14 per cent, 40 x 4.3 (35–47 x 4–4.7)μ

On hard oat agar, culture twenty-four days old; conidia from a sporodochium:

Conidia: 3–septate, 64 per cent, 39.5 x 4.3 (36–52 x 4–4.7)μ
4–septate, 28 per cent, 41 x 4.3 (38–45 x 4–4.7)μ
5–septate, 8 per cent, 43 x 4.5 (38–46 x 4.1–4.7)μ

Average of the above measurements:

Conidia: 0–septate, 16 per cent, 9.5 x 2.25μ
1–septate, 3 per cent, 13.4 x 2.5μ
2–septate, rare
3–septate, 63 per cent, 34.7 x 4.4μ
4–septate, 15 per cent, 37.9 x 4.43μ
5–septate, 3 per cent, 39.35 x 4.55μ

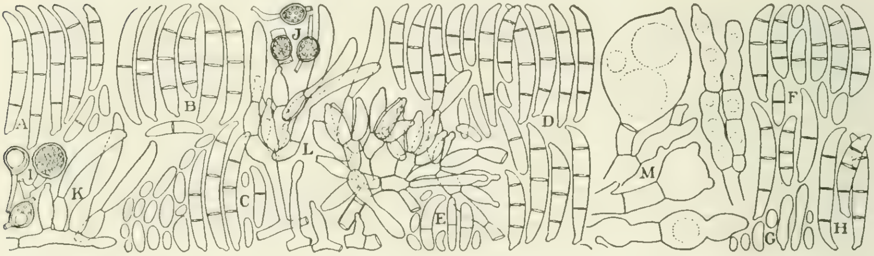

FIG. 34.—*Fusarium sclerotioides var. brevius.* A, *Pseudopionnotal conidia from 8-days-old culture on hard lima-bean agar;* B, *sporodochial conidia,* C, *conidia from aërial mycelium, from 19-days-old culture on slightly acidified hard potato agar;* D, *sporodochial conidia from 45-days-old culture on red raspberry cane plug;* E, *conidia from aërial mycelium from 13-days-old culture on hard lima-bean agar with 2 per cent glucose;* F, *sporodochial conidia,* G, *aërial conidia, from 60-days-old culture on whole steamed potato tuber;* H, *sporodochial conidia from 35-days-old culture on oats;* I, *chlamydospores from 45-days-old culture on red raspberry cane plug;* J, *chlamydospores from 25-days-old culture on corn agar;* K, L, *conidiophores from 19-days-old culture on slightly acidified hard potato agar;* M, *swollen tips of aërial hyphæ from 13-days-old culture on hard lima-bean agar with 2 per cent glucose*

39. *Fusarium sclerotioides* var. **brevius** n. var. (Fig. 34; Pl. II, figs. 1 and 2)

Differs from *F. sclerotioides* mainly by nearly constant absence of 4– and 5–septate conidia, by absence of large bluish black sclerotia, and by noticeably shorter, 3–septate conidia.

Hab. In discolored fibrovascular bundles of tubers of *Solanum tuberosum*, Alabama.

Measurements of the conidia on different media are as follows:

On red raspberry cane plug, culture forty-five days old; conidia from a plectenchymic sporodochium:

Conidia: 0–septate, 8 per cent, 15 x 3 (6–21 x 2.5–3.5)μ
1–septate, 17 per cent, 18 x 3.7 (17–23 x 3.5–4)μ
2–septate, 15 per cent, 20 x 4 (17–23 x 3.5–4.4)μ
3–septate, 60 per cent, 28.3 x 4.3 (19–39 x 3.5–4.8)μ
4–septate, rare

On slightly acidified hard potato agar, culture nineteen days old; conidia from a small sporodochium:

Conidia: 0–septate, rare
1–septate, rare
2–septate, 2 per cent, 24 x 4.2 (20–27 x 3.5–4.7)μ
3–septate, 98 per cent, 30.3 x 4.5 (21–37 x 4.1–4.8)μ

On hard lima-bean agar with 2 per cent glucose, culture thirteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 98 per cent, 7.5 x 2.6 (4.3–18 x 2.3–3.7)μ
1–septate, 2 per cent, 13.5 x 3.2 (10–18 x 2.4–3.5)μ

On same medium as above, culture sixty-five days old; conidia from a sporodochium:

Conidia: 0–septate, 8 per cent, 6.4 x 2.8 (4.4–11 x 2.3–4.1)μ
1–septate, 5 per cent, 22 x 3.8 (15–29 x 3–4.3)μ
2–septate, 2 per cent, 24 x 4 (18–28 x 3.5–4.4)μ
3–septate, 85 per cent, 28 x 4.3 (24–35 x 3.7–4.8)μ

On corn meal agar, culture one hundred and seventy-three days old:

Chlamydospores: 0–septate, in conidia, 7.9 x 7 (5–11.5 x 5–9)μ
1–septate, in mycelium, 13.3 x 8.1 (11–18 x 7–9)μ

On hard lima-bean agar, culture eight days old; conidia from aërial mycelium:

Conidia: 0–septate, 55.5 per cent, 7.8 x 2.8 (5.2–13 x 2.1–3.9)μ
1–septate, 4 per cent, 13.3 x 3.2 (12–15 x 3–3.6)μ
2–septate, 0.5 per cent
3–septate, 40 per cent, 30.8 x 4.1 (23–37 x 3.9–4.2)μ

On same medium as above, culture twelve days old; conidia from pseudopionnotes:

Conidia: 0–septate, 12 per cent
1–septate, 3 per cent
2–septate, 2 per cent
3–septate, 83 per cent, 31 x 4.3 (22–41 x 4–4.7)μ
4–septate, exceptional, 41 x 4.4μ (only one measured)

On hard oat agar, culture twenty-four days old; conidia from a sporodochium:

3–septate, 100 per cent, 31.5 x 4.4 (23–35 x 3.8–4.7)μ

Average of the above measurements:

Conidia: 0–septate, 30.5 per cent, 9.2 x 2.8μ
1–septate, 5 per cent, 16.5 x 3.5μ
2–septate, 3.5 per cent, 22 x 4μ
3–septate, 61 per cent, 29.8 x 4.3μ
4–septate, very rare, 41 x 4.4μ

40. *Fusarium oxysporum* Schlecht.[48] (Figs. 1N$_1$ and 35A; Pl. v, fig. 6)

Schlechtendal, Fl. Berol., **2**:139. 1824. Smith, E. F., and Swingle, D. B., U. S. Plant Indus. Bur., Bul. 55. 1904. Wollenweber, H. W., Phytopath. **3**:28, fig. 1F. 1913. Wollenweber, H. W., Journ. Agr. Research **2**:268. 1914.

Microconidia gradually pointed toward apex, nearly cylindrical in middle half of their length, typically not broader toward apex, usually somewhat distinctly pedicellate, 3-septate dominant, 30.4 x 4.2 (27.5–34 x 4–4.4)μ,[49] in sporodochia and pseudopionnotes; 4–septate macroconidia frequently, and 5 septate ones rarely, present; in mass usually of pinkish buff color; aërial mycelium typically well developed, of medium height (from 3 to 5 millimeters) and density, from white to (in spots on boiled rice) congo pink;

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[48] Original description of *F. oxysporum* Schlecht. (see von Schlechtendal, Flora Berolinensis **2**:139) — "F stroma convexum erumpens varium roseum superficie inaequali rugulosa, sporidiis parvis curvatis utrinque acutissimis "— is insufficient for identification of the species, and, short as it is, it sooner suggests some other species and not that of Smith and Swingle. For a detailed discussion of the matter see Appel and Wollenweber (1910: 144–146).

Wollenweber (1913 a: 28) gives his own description of *F. oxysporum* Schlecht., which, as he states (page 42 of reference cited), " includes some additions to the descriptions given by Smith and Swingle."

For the reason that, at least among pathologists and mycologists of the United States, this organism is fairly well known under this name, and after Wollenweber's description of it the meaning became definite and recognizable, the name *F. oxysporum* is retained here and is used for the organism described by Wollenweber without consideration of Schlechtendal's original description; that is, *F. oxysporum* here is as emended by Wollenweber.

[49] Wollenweber (1913 a: 28) gives the size variation as 25–45 x 3.25–4.5μ. Whether this is average or individual size variation is not stated.

substratum, on potato agar rich in glucose, vinaceous lilac, varying from colorless and orange vinaceous to pomegranate purple and vinaceous purple; plectenchymic sporodochia common on most of the media; bluish black sclerotia (up to 3 millimeters in diameter) constantly present on potato tuber plug and sometimes on different agars.

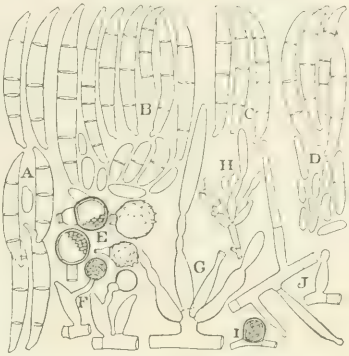

Fig. 35.— a, *Fusarium oxysporum, sporodochial conidia*
b–j, *F. oxysporum var. asclerotium:* b, *Conidia from 14-days-old culture on slightly acidified hard potato agar;* c, *conidia from 46-days-old culture on red raspberry cane plug;* d, *conidia from 7-days-old culture on hard lima-bean agar;* e, *chlamydospores from 46-days-old culture on red raspberry cane plug;* f, *terminal chlamydospores produced on normal conidiophores,* g *and* h, *conidiophores* (h *magnified 250 times*) *from 14-days-old culture on slightly acidified hard potato agar;* i, *chlamydospores from 25-days-old culture on corn agar;* j, *conidiophores from 7-days-old culture on hard lima-bean agar*

Hab. In fibrovascular bundles of diseased stems and tubers of *Solanum tuberosum,* in the United States, perhaps also in Europe, Africa, and other regions, also on *Lycopersicum esculentum* Vigna, and Pisum.

The organism was isolated alone and in association with several other Fusaria, several times from various localities in New York and in other States. The description given above is based on the study of the culture obtained through the courtesy of Dr. Wollenweber.

Measurements of the conidia are as follows:

On slightly acidified hard potato agar, culture nineteen days old; conidia from aërial mycelium close to substratum:

Conidia: 0–septate, 87 per cent, 8 x 2.9 (4.5–12 x 2–4)μ
1–septate, 6 per cent, 16.5 x 3.4 (11–23 x 2.5–4.1)μ
2–septate, 1 per cent, 18.5 x 3.9 (17–26 x 3–4.2)μ
3–septate, 5 per cent, 27.5 x 4 (15–39 x 3–4.7)μ
4–septate, 1 per cent, about 40 x 4.5 (36–44 x 4.3–4.8)μ

On hard lima-bean agar, culture twelve days old; conidia from aërial mycelium:

Conidia: 0–septate, 99 per cent, 6.5 x 2.6 (4.5–16 x 1.75–3.5)μ
1–septate, 1 per cent, 14 x 3.2 (10–17 x 3–3.5)μ

On same medium as above, culture sixty-five days old:
(1) Conidia from a sporodochium
Conidia: 0–septate, 3 per cent
1–septate, 1 per cent
3–septate, 93 per cent, 33.8 x 4.3 (22–42 x 4–4.7)μ
4–septate, 3 per cent, 35.7 x 4.3 (33–41 x 4.1–4.7)μ
5–septate, rare, 35 x 4.4μ (only two measured)
(2) Chlamydospores, terminal and intercalary, 0–septate dominant
Chlamydospores: 0–septate, 8.7 x 8 (7–12.5 x 7–12.5)μ
1–septate, 15.5 x 9.2 (12–22 x 7.7–14)μ
Also in short chain, 2– and 3–septate, 2–septate commoner

On hard oat agar, culture twenty-three days old; conidia from a sporodochium:
Conidia: 3–septate, 93 per cent, 30 x 4.4 (26–35 x 3.9–4.7)μ
4–septate, 6 per cent, 34.5 x 4.5 (31–39 x 4.2–4.7)μ
5–septate, 1 per cent

On corn meal agar, culture one hundred and seventy-three days old; chlamydospores abundant, terminal and intercalary:
Chlamydospores: 0–septate, in conidia, 7.2 x 6.1 (6–9 x 4.8–8)μ
0–septate, in mycelium, 8.6 x 8 (7–10 x 7–11)μ
1–septate, often observed

On hard lima-bean agar, culture fifty days old; chlamydospores intercalary and terminal, in conidia and in mycelium, the latter most commonly observed:
Chlamydospores: 0–septate dominant, 8.9 x 7.3 (5.2–11 x 5.2–11)μ

Average of the above measurements of macroconidia:
Conidia: 3–septate, 0–93 per cent, 30.4 x 4.2μ
4–septate, 0–6 per cent, 36.7 x 4.43 (34.5–60 x 4.3–4.5)μ
5–septate, 0–rare, about 35 x 4.4μ

41. *Fusarium oxysporum* Schlecht. var. **asclerotium** n. var. (Fig. 35, B to J; Pl. v, fig. 7)

Differs from *F. oxysporum* mainly by absence of sclerotia and of definite plectenchymic sporodochia; differs also in color of mycelium and in somewhat longer and narrower macroconidia.

Hab. In rotted tuber of *Solanum tuberosum*, Atlanta, New York.

The organism was isolated from a flexible, semi-soft, rotted potato tuber late in the spring. No other organisms were associated with it. Very similar organisms were isolated also from discolored fibrovascular bundles of potato tubers from various States.

Average measurements of the conidia from four different cultures are as follows:

Conidia: 0–septate, 52.5 per cent, 7.9 x 2.6μ
1–septate, 8 per cent, 15 x 3.1μ
2–septate, rare
3–septate, 39 per cent, 34 x 4 (32–36 x 3.8–4.2)μ[50]
4–septate, 0.5 per cent, 37.5 x 4.35μ
5–septate, very rare, 37.5 x 4.35μ

Chlamydospores (on corn meal culture one hundred and seventy-three days old):

0–septate, in conidia, 7.7 x 7 (6.5–11 x 5–8)μ
0–septate, in mycelium, 9 x 8 (7–11 x 5–8)μ
1–septate, in mycelium, 14 x 9.5 (11–16.5 x 7–13.4)μ[51]

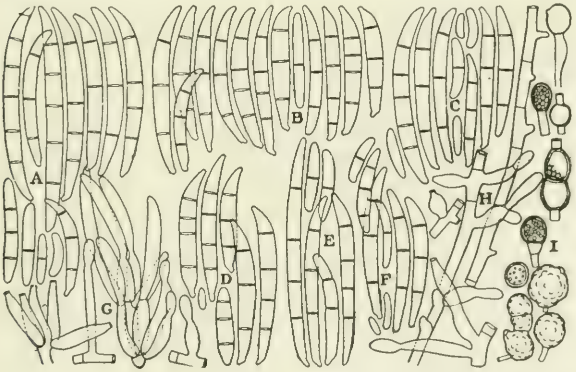

FIG. 36.—*Fusarium oxysporum var. longius.* A, *Pseudopionnotal conidia from 21-days-old culture on slightly acidified hard potato agar;* B, *conidia from 46-days-old culture on red raspberry cane plug;* C, *pseudopionnotal conidia from 5-days-old hard lima-bean agar;* D, *sporodochial conidia from 36-days-old culture on oat agar;* E, *pseudopionnotal conidia from 22-days-old culture on corn agar;* F, *conidia from 202-days-old culture on potato stem plug;* G, *conidiophores from 46-days-old culture on red raspberry cane plug;* H, *conidiophores from 5-days-old culture on hard lima-bean agar;* I, *chlamydospores from 202-days-old culture on potato stem plug*

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[50] Variation of averages of at least ten spore measurements.
[51] Variation of individual spore measurements.

42. *Fusarium oxysporum* Schlecht. var. **longius** n. var. (Figs. 1M$_1$ and 36)
Differs from *F. oxysporum* and other varieties of the species by longer macroconidia, typically 3–septate, 38.94 x 4.04 (34–45 x 3.6–4.4)μ, often 4– and 5–septate; no sclerotia; no macroscopical sporodochia.

Hab. In discolored fibrovascular bundles of tubers of *Solanum tuberosum* and of wilted stems, in New York, California, Maryland, and Connecticut.

On slightly acidified hard potato agar, culture twenty-one days old:

(1) Conidia from pseudopionnotes

Conidia: 0–septate, 1.5 per cent, 10 x 3 (8–12 x 2.5–3.5)μ
1–septate, 3 per cent, 18 x 3.5 (14–21 x 3–4)μ
2–septate, 0.5 per cent
3–septate, 60 per cent, 42.7 x 4.1 (28–54 x 3.5–4.8)μ
4–septate, 26 per cent, 49 x 4.3 (42–58 x 4–4.8)μ
5–septate, 9 per cent, 51.4 x 4.6 (46–60 x 4.3–4.7)μ

(2) Chlamydospores, terminal and intercalary

Chlamydospores: 0–septate, 7–9 x 5–6μ

On red raspberry cane plug, culture forty-six days old; conidia from mycelium close to substratum:

Conidia: 0–septate, 1.7 per cent, 11 x 2.6μ
1–septate, 1 per cent, 19 x 3μ
2–septate, rare
3–septate, 93 per cent, 34 x 4.4 (19–44 x 3.5–5.7)μ
4–septate, 4.3 per cent, 40 x 4.6 (35–44 x 4.2–4.8)μ

On corn meal agar, culture twenty-two days old; conidia from surface of medium:

Conidia: 0–septate, 20 per cent
1–septate, 8 per cent
2–septate, 2 per cent
3–septate, 40 per cent, 45 x 3.6 (36–54 x 3.2–4.2)μ

$$\left.\begin{array}{l}\text{4–septate, 20 per cent}\\ \text{5–septate, 10 per cent}\end{array}\right\}\text{ the largest 67 x 4}\mu$$

On hard lima-bean agar with 2 per cent glucose, culture thirteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 50 per cent, 8.6 x 2.5 (5.2–15 x 2.2–3)μ
1–septate, 10 per cent, 17.5 x 2.9 (12–21 x 2.3–3.5)μ
2–septate, 0.5 per cent, 21 x 3.2μ (only three measured)
3–septate, 30 per cent, 35.6 x 4 (24–50 x 3.2–4.4)μ
4–septate, 8 per cent, 45 x 4.2 (40–51 x 3.9–4.7)μ
5–septate, 1.5 per cent, 47.5 x 4.3 (42–51 x 3.9–5.1)μ

On hard lima-bean agar, culture five days old; conidia from aërial mycelium:

Conidia: 0–septate, 56 per cent, 11 x 2.4μ (only four measured)
1–septate, 4 per cent, 18 x 3.3μ (only four measured)
2–septate, 1.5 per cent
3–septate, 37 per cent, 37 x 4 (26–47 x 3.8–4.4)μ
4–septate, 1 per cent, 43 x 4.3 (40–45 x 4–4.5)μ
5–septate, 0.5 per cent, 50 x 4.4μ (only three measured)

On corn meal agar, culture one hundred and seventy-three days old:
Chlamydospores: (1) 0–septate, in conidia, 8.4 x 6.8 (7–9 x 5–8)μ
(2) 0–septate, terminal, on mycelium, 10 x 9 (9–10.5 x 7.5–10.5)μ

On potato stem plug, culture two hundred and two days old:
(1) Conidia from aërial mycelium close to substratum
Conidia: 0–septate, 40 per cent
1–septate, 20 per cent
2–septate, 10 per cent
3–septate, 30 per cent, 34.3 x 3.9 (22–42 x 3.4–4.1)μ
(2) Chlamydospores, terminal and intercalary, in spores and mycelium
Chlamydospores: 0–septate, 8.9 x 6.8 (6.2–10 x 5.2–9)μ

On hard lima-bean agar, culture twelve days old; conidia from pseudopionnotes:

Conidia: 0–septate, 2 per cent
1–septate, 1 per cent
2–septate, rare
3–septate, 70 per cent, 44 x 4.3 (35–48 x 4–4.8)μ
4–septate, 23 per cent
5–septate, 4 per cent, 46 x 4.3 (40–53 x 4.1–5.2)μ

On hard oat agar, culture fourteen days old; conidia from pseudopionnotes:

Conidia: 0–septate, 4 per cent, 10 x 2.5μ
1–septate, 1 per cent
2–septate, rare
3–septate, 70 per cent, 44 x 4.04 (39–50 x 3.5–4.7)μ
4–septate, 20 per cent, 45 x 4.2 (41–50 x 4–4.7)μ
5–septate, 5 per cent, 49 x 4.46 (42–51 x 4.3–4.7)μ

Average of the above measurements:

Conidia: 0–septate, 24 per cent, 10 x 2.6μ
1–septate, 7 per cent, 17.5 x 3.2μ
2–septate, 1 per cent, 21 x 3.2μ
3–septate, 52 per cent, 38.94 x 4.04μ
4–septate, 12 per cent, 44.25 x 4.35μ
5–septate, 4 per cent, 48.7 x 4.4μ

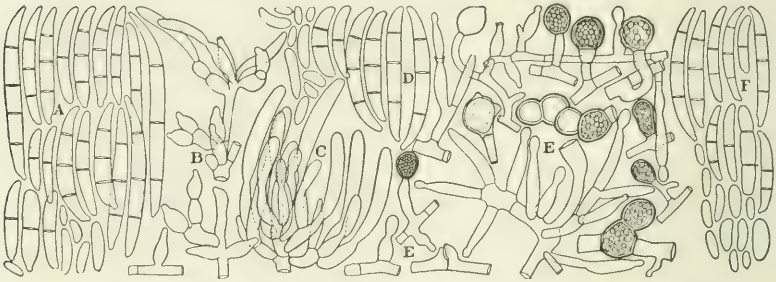

Fig. 37.—*Fusarium oxysporum var. resupinatum.* A, *Pseudopionnotal conidia from 15-days-old culture on slightly acidified hard potato agar;* B, *conidiophores from 6-days-old culture on hard lima-bean agar;* C, *conidiophore from 12-days-old culture on hard lima-bean agar;* D, *pseudopionnotal conidia from 6-days-old culture on hard lima-bean agar;* E, *conidiophores and chlamydospores,* F, *conidia, from 47-days-old culture on red raspberry cane plug*

43. *Fusarium oxysporum* Schlecht. var. **resupinatum** n. var. (Figs. 1L$_1$ and 37; Pl. I, figs. 7 and 8; Pl. V, fig. 5)

Differs from *F. oxysporum* by absence of sclerotia, from var. *asclerotium* and var. *longius* by shorter and somewhat narrower macroconidia. Mycelium on different agars typically entirely resupinate (Pl. I, figs. 7 and 8).

Hab. In discolored fibrovascular bundles of stem and tubers of *Solanum tuberosum*, United States.

This fungus and *F. oxysporum* var. *longius* are the organisms that were found very commonly present in discolored fibrovascular bundles of potato tubers and wilted plants. These organisms are also, as it seems, most commonly recognized by various pathologists as *F. oxysporum* Schlecht., as described by Smith and Swingle (1904); at least cultures of organisms undoubtedly belonging here were received under the name *F. oxysporum* from Mr. Wight in California, Dr. Taubenhaus in Delaware, and Dr. Clinton in Connecticut. The chief difference between these varieties of *F. oxysporum* of Smith and Swingle lies in the absence of sclerotia.

Measurements of spores on different media are as follows:

On slightly acidified hard potato agar, culture fifteen days old; conidia from thin pseudopionnotes:

Conidia: 0–septate, 74 per cent, 8 x 2.7 (5–15 x 2–4.3)μ
1–septate, 9 per cent, 21 x 3 (12–27 x 2.3–4.8)μ
2–septate, 4 per cent, 26 x 3 (20–37 x 2.2–4.1)μ
3–septate, 13 per cent, 35 x 4.2 (22–57 x 3.5–4.7)μ
4–septate, rare, 45 x 4.2μ

On red raspberry cane plug, culture forty-seven days old; conidia from aërial mycelium:

Conidia: 0–septate, 98 per cent, 6.5 x 2.7 (4.5–21 x 2.3–4.3)μ
1–septate, 2 per cent, 18 x 3.2 (12–24 x 2.8–4.1)μ
2–septate, rare, 22 x 3.4 (17–24 x 3–4.1)μ
3–septate, rare, 29 x 3.6 (19–36 x 3–4)μ

On hard lima-bean agar with 2 per cent glucose, culture fifteen days old; conidia from aërial mycelium:

Conidia: 0–septate, 97 per cent, 7.1 x 2.6 (5.2–16 x 1.8–4.3)μ
1–septate, 2 per cent, 16 x 3.1 (10–18 x 2.3–3.8)μ
2–septate, 0.5 per cent
3–septate, 0.5 per cent, 28 x 3.5 (23–36 x 2.4–4)μ

On corn meal agar, culture one hundred and seventy-three days old:

Chlamydospores: 0–septate, in conidia, 7.2 x 7 (5.8–9 x 5–8)μ
0–septate, in mycelium, 9.6 x 8.3 (7–16 x 7–11.5)μ
1–septate, in mycelium, 14.5 x 9.2 (13–18 x 7–11)μ.

On hard lima-bean agar, culture six days old; conidia from pseudopionnotes:

Conidia: 0–septate, 82 per cent
1–septate, 5 per cent
2–septate, 2 per cent
3–septate, 11 per cent, 28 x 4.1 (22–36 x 3.9–4.7)μ

On the same medium as above, culture twelve days old; conidia also from pseudopionnotes;

Conidia: 0–septate, 15 per cent
1–septate, 8 per cent
3–septate, 77 per cent, 33.5 x 4.1 (29–37 x 4–4.5)μ; the smallest 21 x 3.4μ, the largest 40 x 4.5μ (both 3–septate)

Chlamydospores all kinds except terminal, 0–septate dominant, measuring 8.3 x 6.7 (6–10.5 x 5.2–8)μ

Average of the above measurements:

Conidia: 0–septate, 73 per cent, 7.2 x 2.7μ
1–septate, 5 per cent, 18 x 3.1μ
2–septate, 1.5 per cent, 24 x 3.2μ
3–septate, 20.5 per cent, 30.7 x 3.9μ
4–septate, rare, 45 x 4.2μ

X. SECTION DISCOLOR Wr., Phytopath. **3**:31, fig. 1, G, H, J, 1913

Conidia sickle-shaped, at the middle nearly cylindrical or broadened toward the apex, somewhat abruptly apically attenuated, distinctly pedicellate; mostly 3– to 5–septate, 5–septate dominant; microconidia typically absent; chlamydospores intercalary only, usually scant; mycelium typically well developed, with from nearly white to orange color as type. Substratum from nearly colorless to chamois, pomegranate purple, and spectrum red. Color of conidia very variable, mostly cinnamon to orange.

Differs from sections Elegans and Martiella by absence of microconidia, by the conidia being typically somewhat abruptly attenuate, by absence of terminal chlamydospores, and by absence of vinaceous, drab-gray, tawny-olive, and blue color of substratum. By the shape of conidia this section occupies an intermediate position between sections Elegans and Martiella; by its color it is closely related to sections Roseum and Ferruginosum.

44. *Fusarium trichothecioides* Wr. (Figs. 1, U_1 and V_1, and 38; Pl. IV, fig. 8)

Jamieson, C. O., and Wollenweber, H. W., Journ. Washington Acad. Sci. **2**:146–152, fig. 1. 1912.

Syn. *Fusarium tuberivorum* Wilcox and Link, Nebraska Agr. Exp. Sta., Research bul. 1:48. 1913.

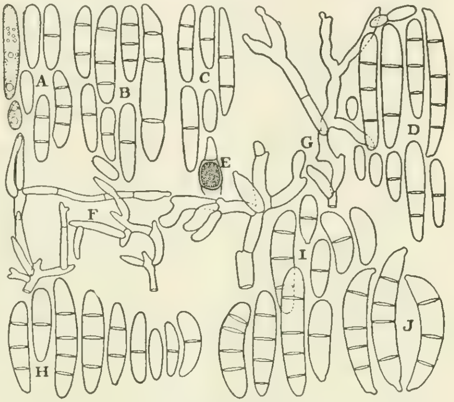

Fig. 38.—*Fusarium trichothecioides.* A, *Conidia from 41-days-old culture on rye straw;* B, *conidia from 70-days-old culture on potato stem plug;* C, *conidia from 73-days-old culture on red raspberry cane plug;* D, *conidia from 15-days-old culture on slightly acidified hard potato agar;* E, *chlamydospore in conidium from 73-days-old culture on red raspberry cane plug;* F, *conidiophores (magnified 250 times) from 70-days-old culture on potato stem plug;* G, *conidiophores from 15-days-old culture on slightly acidified hard potato agar;* H, *conidia from 5-days-old culture on hard lima-bean agar;* I, *comma,* J, *discolor, type of conidia from 5-days-old culture on potato tuber plug*

Conidia of comma and discolor types the former predominating and under ordinary cultural conditions occurring almost exclusively, mostly 1–septate, 16 x 4.6 (14–17 x 4.2–5.4)μ, often 0– to 3–septate, seldom 4– or 5–septate, 6–septate rare; sporodochial conidia sickle-shaped, 3– to 5–septate, 24–42 x 4.5–5.5μ.[52] Typical conidiophores of *comma* stage shown in figure 38. Sporodochial conidiophores similar to those of *F. discolor*. *F. trichothecioides* can be recognized at once by color and appearance of its powdery masses of spores produced on aërial mycelium (Pl. IV, fig. 8). Chlamydospores few and not prominent.

Hab. On rotted tubers of *Solanum tuberosum*, United States.

This species is noticeably different from all other species of the section Discolor. It is listed under this section chiefly because of its resemblance in the sporodochial form. Perhaps it should be made the type of a new section.

The organism, under the name *F. tuberivorum* Wilcox and Link, was extensively studied by Wilcox, Link, and Pool (1913). The identity of *F. tuberivorum* and *F. trichothecioides* Wr. is discussed by Wollenweber (1913 c: 206). In the writer's opinion there is no doubt that this is correct.

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[52] The size of 3– to 5–septate conidia is as given by Wollenweber. The writer isolated this Fusarium from specimens of rotted potato tubers received from North Dakota in July, 1912, and from Texas in December, 1913. No special study of this organism was made at first, because it is easily recognizable even after a superficial examination, and also because its description as given by Wollenweber (see Jamieson and Wollenweber, 1912) is entirely sufficient for its identification.

The few measurements of the conidia on different media are as follows:

On slightly acidified hard potato agar, culture fifteen days old:
Conidia: 0–septate, 23 per cent, 8.2 x 3.8 (5–15 x 3–4.7)μ
1–septate, 59 per cent, 19 x 4.2 (12–26 x 3.5–4.7)μ
2–septate, 8 per cent, 22.4 x 4.3 (16–35 x 4.3–5.2)μ
3–septate, 10 per cent, 28.1 x 5 (20–39 x 4.3–5.3)μ
4– and 5–septate, few, 40–45 x 5μ

On red raspberry cane plug, culture seventy-three days old:
Conidia: 0–septate, 39 per cent, 12 x 4.2 (7–14 x 3–4.8)μ
1–septate, 50 per cent, 14 x 4.3 (14–20 x 3.9–4.8)μ
2–septate, 11 per cent, 22 x 4.5 (19–32 x 4.1–5.2)μ

On potato tuber plug, culture five days old, kept at a low (from 3° to 7° C.) temperature:
Conidia: 0–septate, 6 per cent, 14 x 5.2 (10–17 x 4.8–5.5)μ
1–septate, 53 per cent, 16 x 5.4 (12–23 x 4.4–7)μ
2–septate, 15 per cent
3–septate, 19 per cent, 26 x 5.8 (19–42 x 5.2–7)μ
4–septate, 6 per cent, 35 x 6.2 (33–40 x 5.4–7)μ
5–septate, 1 per cent, 38 x 6.3 (31–52 x 5.4–7)μ
6–septate, exceptional, 41 x 6.3 (39–47 x 6–7)μ (only three measured)

Average of the above measurements:
Conidia: 0–septate, 23 per cent, 11 x 4.4μ
1–septate, 54 per cent, 16 x 4.6μ
2–septate, 11 per cent, 21 x 4.8μ
3–septate, 10 per cent, 27 x 5.4μ
4–septate, 2 per cent
5–septate, rare, 38 x 6.3μ
6–septate, very rare

45. **Fusarium subpallidum** n. sp. (Fig. 39; Pl. v, fig. 12)

Conidia sickle-shaped, typically abruptly constricted at apex, slightly pedicellate to papillate, somewhat broader in the middle, mostly 5–septate, 29.1 x 5.53 (28–32.5 x 5.4–5.8)μ, 3– and 4–septate common, 6– and 7–septate very rare; chlamydospores common, mostly in long chains; aërial mycelium well developed; plectenchymic sporodochia (up to 3 millimeters in diameter) common; color of aërial mycelium from white to sea-foam yellow and honey yellow; color of substratum, on agars rich in glucose, mostly from chamois to raw sienna and antique brown in some old cultures; color of conidia, in mass, commonly from pinkish buff to pale orange, sometimes from green to blue.[53]

Hab. On superficial dry rot of tubers of *Solanum tuberosum*. The organism was isolated from rotted potato tubers received from Edgerton, Louisiana.

Latin description.—Conidiis falciformibus, typice subito apice constrictis, parum pedicellatis demum papillatis, medio aliquatenus latioribus, plerumque 5–septatis, 29.1 x 5.53 (28–32.5 x 5.4–5.8)μ, saepe etiam conidiis 3–4–septatis; conidiis 6–7–septatis rarissimis; chlamydosporis frequentibus plerumque longis catenulatis; aerio mycelio plene maturo; plectenchymicis sporodochiis (0–3 mm. diam.) frequentibus; aerio mycelio ex albo "sea-foam yellow" (R) vel "honey-yellow" (R); substrato, in agaribus perglucosis, plerumque e "chamois" (R) "raw sienna" (R) vel etiam in nonnullis culturis maturis "antique brown" (R); conidiis in totum plerumque e "pinkish buff" (R) pallide "orange" (R) vel interdum e viridi caeruleis.

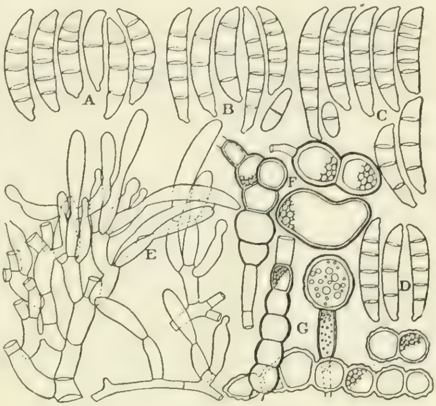

FIG. 39.—*Fusarium subpallidum.* A, *Sporodochial conidia from 70-days-old culture on hard potato agar;* B, *sporodochial conidia from 35-days-old culture on hard oat agar;* C, *conidia from a thin slimy layer from 15-days-old culture on slightly acidified hard potato agar;* D, *conidia from aërial mycelium from 52-days-old culture on red raspberry cane plug;* E, *conidiophores from 15-days-old culture on slightly acidified hard potato agar;* F, *chlamydospores from 175-days-old culture on corn agar;* G, *conidiophores from 52-days-old culture on red raspberry cane plug*

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[53] A very exceptional color for an organism of the section Discolor, and observed only in *F. subpallidum.* When the conidia are from green to blue in mass, some of them appear under the microscope very densely blue as if stained with methyl blue. The same was observed also in the case of *F. coeruleum*, *F. Martii*, and *F. Solani* var. *cyanum.*

Hab. In tuberibus aridis et extra putridis Solani tuberosi. Fungus e tuberibus Solani tuberosi putridis ab Edgerton in Louisiana, Amer. bor. receptis, sejungebatur.

Measurements of conidia on various media are as follows:

On slightly acidified hard potato agar, culture fifteen days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare
1–septate, rare
2–septate, rare
3–septate, 23 per cent, 24.4 x 5.2 (16–30 x 4.7–5.7)μ
4–septate, 31 per cent, 25.6 x 5.5 (19–32 x 5.2–5.9)μ
5–septate, 45 per cent, 28 x 5.7 (24–34 x 4.7–5.9)μ
6– and 7–septate, 1 per cent, 31 x 6.1 (26–35 x 5.7–6.4)μ

On red raspberry cane plug, culture fifty-nine days old; conidia from aërial mycelium:

Conidia: 3–septate, 40 per cent, 24 x 4.7 (20–28 x 4.3–5)μ
4–septate, 18 per cent
5–septate, 42 per cent, 28 x 5.2 (24–31 x 5–5.3)μ (only four measured)

On hard potato agar, culture one hundred and seventy-five days old:

(1) Conidia from a sporodochium

Conidia: 3–septate, 4 per cent, 26 x 5.3 (23–28 x 5.2–5.4)μ
4–septate, 1 per cent
5–septate, 95 per cent, 29 x 5.8 (26–33 x 5.2–6.1)μ

(2) Chlamydospores

0–septate, in conidia, 8 x 7.5 (7–9 x 6–9)μ
1–septate, in mycelium, 15.5 x 10.3 (10–18 x 8.7–13.2)μ, the largest observed being 21 x 14μ

On medium soft potato agar, culture fifty days old:

(1) Conidia from thin pseudopionnotes

Conidia: 3–septate, 28 per cent, 25 x 5.4 (26–29 x 4.8—5.9)μ
4–septate, 23 per cent, about the size of 5–septate
5–septate, 49 per cent, 28 x 5.6 (24–32 x 5.2–6.3)μ

(2) Chlamydospores

0– and 1–septate, in clusters and chains, 7–16μ in diameter

On hard oat agar, culture twenty-three days old; conidia from sporodochium:

Conidia: 5–septate, 100 per cent, 32.5 x 5.4 (24–35 x 5.2–5.7)μ

Average of the above measurements:

Conidia: 0– to 2–septate, rare
3–septate, 19 per cent, 24.5 x 5.2μ
4–septate, 15 per cent, 25.6 x 5.5μ
5–septate, 66 per cent, 29.1 x 5.53μ
6– and 7–septate, very rare, 31 x 6.1μ

46. *Fusarium subpallidum* var. **roseum** n. var. (Fig. 1, S_1 and T_1; Pl. IV, fig. 3)

Differs from *F. subpallidum* by lower septation of conidia, the majority being 3–septate, 25.25 x 4.9 (22.5–27 x 4.7–5)μ, and by a tint or shade of from Tyrian rose to pomegranate purple in substratum on agars rich in glucose.

Hab. On rotted tubers of *Solanum tuberosum*, Kentucky.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture fifteen days old; conidia from pseudopionnotes:

Conidia: 0–septate, few
1–septate, 1 per cent, 17.5 x 4.7 (10–18 x 4.7)μ
2–septate, 1 per cent
3–septate, 44 per cent, 24.5 x 4.8 (18–29 x 4.7–5.3)μ
4–septate, 35 per cent, 27 x 4.9 (24–30 x 4.7–5.3)μ
5–septate, 19 per cent, 29 x 5.3 (24–32 x 4.7–5.7)μ

On red raspberry cane plug, culture seventy-three days old; conidia from a sporodochium:

Conidia: 0–septate, exceptional

$$\left.\begin{array}{l}\text{1–septate}\\ \text{2–septate}\end{array}\right\}\text{ 1 per cent }\left\{\begin{array}{l}12 \text{ x } 4.1\ (10\text{–}14 \text{ x } 3.8\text{–}4.4)\mu\\ 23 \text{ x } 4.8\mu\ \text{(only a few measured)}\end{array}\right.$$

3–septate, 34 per cent, 26 x 5 (21–28 x 4.7–5.3)μ
4–septate, 35 per cent, 28 x 5.3 (23–30 x 4.8–5.7)μ
5–septate, 30 per cent, 30 x 5.3 (24–33 x 5–5.7)μ

On hard lima-bean agar, culture thirty-two days old; conidia from a sporodochium:

Conidia: 1–septate, 16 per cent
2–septate, 7 per cent
3–septate, 77 per cent, 22.5 x 4.8 (18–25 x 4.7–5.3)μ
4–septate, few, 26 x 5.1 (25–30 x 4.7–5.3)μ

On hard oat agar, culture twenty-three days old; conidia from a sporodochium:

Conidia: 3–septate, 90 per cent, 27 x 4.7 (24–34 x 4.1–4.9)μ
4–septate, 9 per cent, 29 x 4.9 (24–34 x 4.1–5.2)μ
5–septate, 1 per cent, 33 x 4.9 (30–40 x 4.8–5.2)μ

Average of the above measurements:

Conidia: 0–septate, rare
1–septate, 4.5 per cent, 17.5 x 4.7μ
2–septate, 2 per cent
3–septate, 61 per cent, 25.25 x 4.9μ
4–septate, 20 per cent, 27.5 x 5.1μ
5–septate, 12.5 per cent, 29.5 x 5.3μ

47. **Fusarium clavatum** n. sp. (Figs. 1R$_1$ and 40; Pl. III, figs. 11 and 12; Pl. VII, fig. 2)

Conidia sickle-shaped, typically distinctly broader in upper third of their length, somewhat suddenly constricted at the apex, slightly pedicellate, mostly 3- to 5-septate, 5-septate measuring 36.2 x 5.05 (32–46 x 4.8–5.2)μ; aërial mycelium of a medium development (2 to 4 millimeters high, more or less loose) to nearly absent, and then substratum covered with pseudopionnotes; chlamydospores scant, not in long chains; color of aërial mycelium and substratum from white to light pink and that shown on Plate III, figures 11 and 12; color of pseudopionnotes from pale pink to deep olive-buff and chocolate brown (Pl. III, fig. 11); color of sporodochia bright orange (Pl. VII, fig. 2).

Hab. On rotted tubers of *Solanum tuberosum*, together with *F. coeruleum*, Castile, New York.

Latin description.—Conidiis falciformibus, typice distincte latioribus in superiore tertio longitudinis, apice aliquatenus subito constrictis, parum pedicellatis, plerumque 3–5–septatis, 5–septatis, 36.2 x 5.05 (32–46 x 4.8–5. 2)μ; aerio mycelio mediocriter maturo (2–4 mm. alt., plus minusve laxo) vel ferme nullo, quae cum ita sint substrato pseudopionnotibus obsito; chlamydosporis paucis, non catenulatis; aerio mycelio substratoque ex albo pallide rubello vel eodem colore quod in Tab. III, figg. 11, 12, exhibente; pseudopionnotibus e pallide rubello "olive-buff" (R) et "chocolate brown" (R); sporodochiis nitide "orange" (R).

Hab. In tuberibus putridis Solani tuberosi una cum F. coeruleo, Castile, New York, Amer. bor.

Measurements of conidia on various media are as follows:

On red raspberry cane plug, culture ninety-two days old; conidia from pseudopionnotes:

Conidia: 3–septate, 5 per cent, 27 x 4.2 (20–35 x 4–4.7)μ
5–septate, 95 per cent, 35 x 4.8 (29–39 x 4.3–5.7)μ

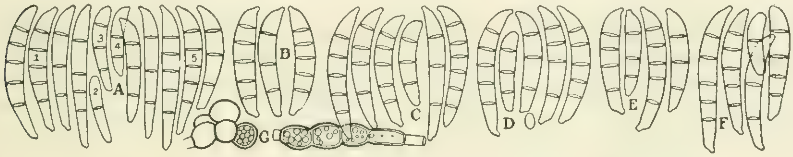

FIG. 40.—*Fusarium clavatum.* A, *Conidia from a thin layer near substratum from 92-days-old culture on red raspberry cane plug (conidia 1 and 5 are typical, but in certain areas conidia like 2, 3, and 4 were present almost to the exclusion of the other kinds);* B, *conidia from thin layer from 120-days-old culture on potato stem plug;* C, *pseudopionnotal conidia from 41-days-old culture on rye straw;* D, *pseudopionnotal conidia from 32-days-old culture on hard oat agar;* E, *pseudopionnotal conidia from 10-days-old culture on potato agar;* F, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar;* G, *chlamydospores from 10-days-old culture on potato agar*

On hard lima-bean agar with 2 per cent glucose, culture twenty-two days old; conidia from aërial mycelium:

Conidia: 3–septate, 60 per cent, 26 x 4.9 (19–38 x 4.3–5.3)μ
4–septate, 16 per cent, 32 x 5.2μ (only one measured)
5–septate, 24 per cent, 32 x 5.15 (29–36 x 4.7–5.3)μ

On medium soft potato agar, culture ten days old; conidia from aërial mycelium (chlamydospores intercalary, in chains and clusters):

Conidia: 1–septate, 1 per cent, 16 x 4.4μ (only three measured)
3–septate, 82 per cent, 26 x 5 (23–31 x 4.7–5.4)μ
4–septate, 9 per cent, 29 x 5.1 (28–32 x 4.7–5.3)μ
5–septate, 8 per cent, 32 x 5.2 (29–34 x 4.7–5.3)μ

On hard lima-bean agar, culture nine days old; conidia from aërial mycelium close to substratum:

Conidia: 1–septate, very rare
2–septate, very rare
3–septate, 33 per cent, 30 x 4.6 (19–34 x 4.3–4.8)μ
4–septate, 23 per cent
5–septate, 44 per cent, 36 x 5 (33–40 x 4.6–5.4)μ

On same medium as above, culture fifteen days old; conidia from pseudopionnotes:

Conidia: 2–septate, very rare
3–septate, 36 per cent, 33 x 5 (22–39 x 4.6–5.3)μ
4–septate, 22 per cent
5–septate, 42 per cent, 36.5 x 5.1 (33–41 x 4.9–5.3)μ

Average of the above measurements:

Conidia: 1–septate, rare, 16 x 4.4μ
2–septate, very rare
3–septate, 43 per cent, 28.5 x 4.75μ
4–septate, 15 per cent
5–septate, 42 per cent, 36.2 x 5.05μ

48. *Fusarium discolor* Ap. et Wr. (Fig. $1Q_1$ and 41; Pl. IV, fig. 4; Pl. V, fig. 11)

Appel, O. and Wollenweber, H. W., Arb. K. biol. Anst. Land– u. Forstw. **8**:108–115; Pl. I, figs. 50 to 59; Pl. III, fig. 7. 1910.

Conidia for greater part of their length of nearly even diameter, sickle-shaped, gradually attenuated, often somewhat suddenly constricted at the apex, pedicellate, mostly 3– to 5–septate, 5–septate measuring 38.7 x 5.1 (35–40 x 4.6–5.1)μ.[54] on aërial mycelium, in pseudopionnotes, and in plectenchymic sporodochia; chlamydospores scant, not in long chains; aërial mycelium from poorly to well developed, from pale pink-buff to ochraceous orange and Eugenia red; color of substratum, on agars rich in glucose, from pale salmon at an early stage and warm sepia in old cultures to tyrian and ox blood; color of conidia mostly from light ochraceous salmon to ochraceous buff.

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[54] Size of 5–septate conidia according to Wollenweber's data (see Appel and Wollenweber 1910 : 111–112) is 36 x 5.1 (29–39 x 4.5–5.5)μ. The writer's measurements were taken from cultures of the same organism. Average size of *F. discolor* strain isolated in United States is, for 3–septate conidia, 30.2 x 4.4μ (from 25 to 98 per cent), and for 5–septate conidia, 38.4 x 4.85 (33.5–44 x 4.5–5)μ (from 0 to 53 per cent).

Hab. In stems and rotten tubers of *Solanum tuberosum*, in Germany and in the United States.

Differs from *F. clavatum* mainly by indistinctly clavate conidia and by presence of denser hues of red color in substratum.

Measurements of conidia on various media are as follows:

On slightly acidified hard potato agar, culture twenty-four days old; conidia from pseudopionnotes:

Conidia: 1–septate, rare
3–septate, 11 per cent, 32.3 x 4.3 (27–37 x 4.1–4.9)μ
4–septate, 14 per cent, 36 x 4.5 (32–42 x 4.3–5)μ
5–septate, 75 per cent, 39.2 x 4.7 (31–46 x 4.3–5.7)μ
6–septate, few, 53 x 5.8μ (only one measured)

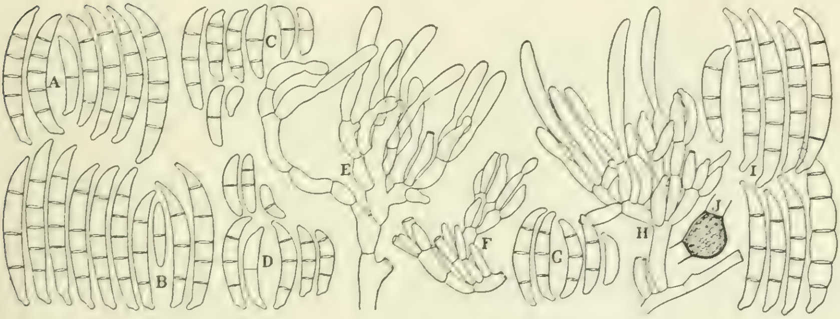

FIG. 41.— *Fusarium discolor.* A, *Conidia from a small plectenchymic sporodochium;* B, *conidia from pseudopionnotes from 15-days-old culture on slightly acidified hard potato agar;* C, *conidia from 81-days-old culture on red raspberry cane plug;* D, *conidia from 36-days-old culture on potato stem plug;* E, *conidiophores from aërial mycelium from 81-days-old culture on red raspberry cane plug;* F, *conidiophores from minute sporodochia from 36-days-old culture on potato stem plug;* G, *conidia from mycelial growth from 41-days-old culture on rye straw;* H, *conidiophore from pseudopionnotes from 15-days-old culture on slightly acidified hard potato agar;* I, *pseudopionnotal conidia from 9-days-old culture on hard lima-bean agar;* J, *intercalary chlamydospore from culture on corn agar*

On red raspberry cane plug, culture seventy-two days old; conidia from minute sporodochia on aërial mycelium:

Conidia: 1– and 2–septate, rare
3–septate, 27 per cent, 30 x 4.4 (19–49 x 3.7–4.8)μ
4–septate, 24 per cent, 36 x 4.6 (26–49 x 3.9–5.9)μ
5–septate, 49 per cent, 40 x 5.2 (32–50 x 4.9–5.9)μ

On potato tuber plug, culture ninety-nine days old; conidia from a sporodochium:

Conidia: 3–septate, 6 per cent, 26 x 4.7 (22–30 x 4.3–4.8)μ
4–septate; 25 per cent, 33 x 5 (28–40 x 4.6–5)μ
5–septate, 69 per cent, 35 x 5.2 (28–42 x 4.7–5.7)μ

On hard lima-bean agar with 2 per cent glucose, culture fifty-four days old; conidia from a small sporodochium:

Conidia: 1– to 3–septate, rare
4–septate, 2 per cent, 36 x 4.9μ (only two measured)
5–septate, 98 per cent, 40 x 5.2 (33–44 x 4.6–5.5)μ

On hard lima-bean agar, culture thirty-four days old; conidia from pseudopionnotes:

Conidia: 1–septate, very rare
3–septate, 49 per cent, 33.5 x 4.8 (24–44 x 4.1–6)μ
4–septate, 31 per cent
5–septate, 20 per cent, 39.3 x 5.1 (31–47 x 4.6–6.2)μ

Average of the above measurements:

Conidia: 1–septate, very rare
2–septate, very rare
3–septate, 19 per cent, 30.5 x 4.55μ
4–septate, 19 per cent
5–septate, 62 per cent, 38.7 x 5.1μ
6–septate, very rare, 53 x 5.8μ

49. *Fusarium discolor* Ap. et Wr. var. *sulphureum* (Schlecht.) Ap. et Wr. (Pl. IV, fig. 11)

Cf. Schlechtendal, Fl. Berol. **2**:134. 1824. Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. **8**:115–118. 1910.

Differs from *F. discolor* by absence of red color in mycelium and substratum, and by entirely exposed pseudopionnotes on various agars, a characteristic culture of which is shown in Plate IV, figure 11.

Average percentage of different septation types and sizes of conidia on three different media are as follows:

Conidia: 1–septate, about 0.5 per cent
2–septate, very rare
3–septate, about 25 per cent, 28.5 x 4.2μ

4–septate, about 28 per cent
5–septate, about 46.5 per cent, 40 x 4.9 (38–41 x 4.8–4.9)μ
6–septate, rare, 48 x 5.2μ

50. *Fusarium discolor* Ap. et Wr. var. **triseptatum** n. var. (Figs. 1w_1 and 42; Pl. IV, figs. 5 and 6; Pl. V, fig. 10)

Differs from *F. discolor* by dominance of 3–septate conidia 24.2 x 4.7 (22–26 x 4.5–4.9)μ, by presence of very large (up to 1.2 centimeters in diameter), warty, plectenchymic bodies (producing conidia or remaining sterile) of a pale pinkish buff with spots of a darker color, by more intense color of mycelium and substratum (see Plate IV, figures 5 and 6), and by production of larger sporodochia with darker spore masses, much the same as shown for *F. culmorum* var. *leteius* in Plate IV, figure 10.

Hab. On rotted tubers of *Solanum tuberosum* together with *F. coeruleum*, Long Island, New York.

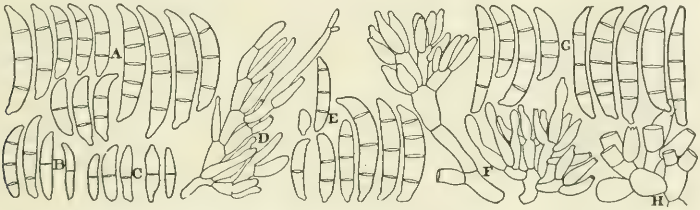

FIG. 42.—*Fusarium discolor var. triseptatum.* A, *Conidia from minute aërial sporodochia from 22-days-old culture on slightly acidified hard potato agar;* B, *conidia from 66-days-old culture on potato stem plug;* C, *conidia from aërial mycelium from culture on rye straw;* D, *conidiophore from aërial mycelium from 66-days-old culture on potato stem plug;* E, *conidia from plectenchymic sporodochia,* F, *conidiophores, from 22-days-old slightly acidified hard potato agar;* G, *conidia from plectenchymic sporodochia from 9-days-old culture on hard lima-bean agar;* H, *basal part of aërial compound conidiophore from 73-days-old culture on red raspberry cane plug*

Measurements of conidia on a few different media are as follows:

On slightly acidified hard potato agar, culture twenty-two days old; conidia from minute sporodochia on aërial mycelium:

Conidia: 0–septate, rare
1–septate, 1.6 per cent, 16 x 3.9μ
2–septate, 6.4 per cent, 18.5 x 4.3 (17–21 x 3.9–4.7)μ
3–septate, 91.2 per cent, 22.5 x 4.5 (17–39 x 3.9–5.7)μ
4–septate, 0.8 per cent
5–septate, very rare, 31 x 5.3 (28–35 x 5.2–5.8)μ

On red raspberry cane plug, culture seventy-three days old; conidia from a sporodochium:

Conidia: 0–septate, very rare
1–septate, 2 per cent, 15 x 3.7 (10–20 x 3–4.2)μ
2–septate, 8 per cent, 20 x 4.1 (17–23 x 3.6–4.7)μ
3–septate, 88 per cent, 24 x 4.6 (17–29 x 3.9–5.3)μ
4–septate, 2 per cent, 28 x 5.2 (25–30 x 4.8–5.7)μ

On hard lima-bean agar, culture nine days old; conidia from a young plectenchymic sporodochium:

Conidia: 1–septate, 2 per cent
2–septate, 3 per cent, 20 x 4.5 (13–25 x 4.1–4.7)μ
3–septate, 88 per cent, 26 x 4.9 (18–33 x 4.2–6)μ
4–septate, 6 per cent
5–septate, 1 per cent, 30 x 5.8μ (only a few measured)

Average of the above measurements:

Conidia: 0–septate, very rare
1–septate, 2 per cent, 15.5 x 3.8μ
2–septate, 6 per cent, 19.5 x 4.3μ
3–septate, 89 per cent, 24.2 x 4.7μ
4–septate, 3 per cent, 29.5 x 5.25μ
5–septate, few, 30.5 x 5.55μ

The organism seems to be so different from *F. discolor* that one may wonder why it is considered as a variety rather than an independent species, especially in view of the fact that there are some organisms designated as species which apparently differ very slightly from the other related species. The explanation lies mainly in the fact that, notwithstanding a seemingly great difference, this difference is in unstable characters — greater proportion of one type of septation instead of another, denser color, larger sporodochia, and so forth — and, moreover, in certain instances the two organisms approach each other so closely as to be distinguished only with considerable difficulty if at all.

51. *Fusarium culmorum* (W. Smith) Sacc. (Pl. IV, fig. 9; Pl. V, fig. 8)

Cf. Wollenweber, H. W., Journ. Agr. Research **2**:260–261, Pl. XVI, fig. J. 1914.

Syn. *Fusarium culmorum* W. G. Smith, Dis. Field and Gard. Crops, pp. 208–210, fig. 92. 1884.
Fusarium Schribauxi Delacr., Bul. Soc. Mycol. France **6**:99, pl. 15, fig. 1. 1890. Saccardo, Syll. Fung. **10**:726. 1890.
Fusarium culmorum (W. Sm.) Sacc., Syll. Fung. **11**:651. 1895.
Fusarium corallinum Mattirolo (non Sacc.), Mem. R. Accad. Sci. Ist. Bologna, ser. 5:**6**: 677, figs. 16 and 17. 1897.
Fusarium rubiginosum Ap. et Wr., Arb. K. biol. Anst. Land- u. Forstw. 8:95–108; text fig. 8; Pl. I, figs. 31 to 48. 1910.

Conidia for a greater part of their length of an even diameter, mostly 5-septate, 38.5 x 5.85 (37–40 x 5.3–6.2)μ, somewhat suddenly constricted at apex; pedicellate, of distinctly ochraceous orange color under microscope; chlamydospores of more or less common occurrence in mycelium and in conidia, not in long chains; aërial mycelium well developed, high (up to 1 centimeter and more), very loose, at first from white to pinkish cinnamon, and then to jasper and Eugenia red; substratum, on potato agar rich in glucose, of from spectrum red to carmine-pomegranate purple, with more or less brick red color; color of conidia in mass from cinnamon and light ochraceous to mikado brown and warm sepia; sporodochia minute, separate or converging into pseudopionnotes.

Hab. On cereals and on potato tubers and some other hosts, in Europe and the United States.

The organism was isolated by the writer from rotted potato tubers, alone and in association with other Fusaria.

Measurements of conidia on different media are as follows:[55]

On slightly acidified hard potato agar, culture twenty-three days old; conidia from pseudopionnotes:

Conidia: 3-septate, 5 per cent, 33 x 5.9 (21–36 x 4.7–6.1)μ
4-septate, 10 per cent, 35 x 6.1 (30–37 x 5.7–6.4)μ
5-septate, 85 per cent, 37 x 6.2 (32–46 x 5.2–6.5)μ

On red raspberry cane plug, culture seventy-two days old:

(1) Conidia from a sporodochium

Conidia: 3-septate, 3 per cent
4-septate, 10 per cent
5-septate, 87 per cent, 40 x 6.2 (33–53 x 5.8–7)μ

---

[55] Average of the measurements given by Appel and Wollenweber (1910:106) is as follows:
Conidia: 0- to 2-septate, about 1 per cent
3-septate, about 11 per cent, 26 x 5.5μ
4-septate, about 13 per cent, 27 x 5.7μ
5-septate, about 74 per cent, 39 x 6.1μ
6-septate, about 1 per cent, 45 x 6μ

(2) Conidia from aërial mycelium
Conidia: 3–septate, 60 per cent, 30 x 4.7 (19–39 x 4.1–5.9)μ
4–septate, 25 per cent, 37 x 5 (31–42 x 4.4–5.9)μ
5–septate, 15 per cent, 39 x 5.3 (35–43 x 5.2–5.9)μ

On hard lima-bean agar with 2 per cent glucose, culture one hundred and fifty-four days old; conidia from pseudopionnotes:
Conidia: 0–septate, rare, 8 x 4μ (only a few measured)
1–septate, rare, 12 x 3.7μ (only a few measured)
2–septate, rare
3–septate, 3 per cent, 26 x 4.7 (17–34 x 4.1–5.5)μ
4–septate, 7 per cent
5–septate, 89 per cent, 38 x 5.7 (33–47 x 5.2–6.1)μ
6–septate, 1 per cent, 49 x 5.7μ (only a few measured)
7–septate, very rare, 56 x 6.1μ (only a few measured)

Average of the above measurements:
Conidia: 0–septate, very rare, 8 x 4μ
1–septate, very rare, 12 x 37μ
2–septate, very rare
3–septate, 18 per cent, 29.67 x 5.1μ
4–septate, 13 per cent
5–septate, 69 per cent, 38.5 x 5.85μ
6–septate, few, 49 x 5.7μ
7–septate, exceptional, 56 x 6.1μ

52. *Fusarium culmorum* (W. Smith) Sacc. var. **leteius** n. var. (Figs. 1D$_2$ and 43; Pl. iv, figs. 1, 2, 10; Pl. v, fig. 9)

Differs from *F. culmorum* mainly by somewhat comma-like conidia from aërial mycelium, by typical presence of medium large (up to from 3 to 5 millimeters in diameter) sporodochia, and by somewhat broader average size of conidia.

Hab. On rotted tubers of *Solanum tuberosum*, Atlanta and Forks, New York.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture fifteen days old; conidia from a sporodochium:

Conidia: 1–septate, 0.5 per cent, 18 x 5.2 (14–22 x 4.7–5.9)μ
2–septate, 5.5 per cent, 23 x 6.9 (20–26 x 6.4–7.9)μ
3–septate, 34 per cent, 26.5 x 7 (21–33 x 5.6–7.6)μ
4–septate, 38 per cent, 33.2 x 7.3 (26–38 x 6.4–7.9)μ
5–septate, 22 per cent, 36.4 x 7.5 (31–44 x 7–8.8)μ
6–septate, very few, 44.5 x 8.9μ (only one measured)

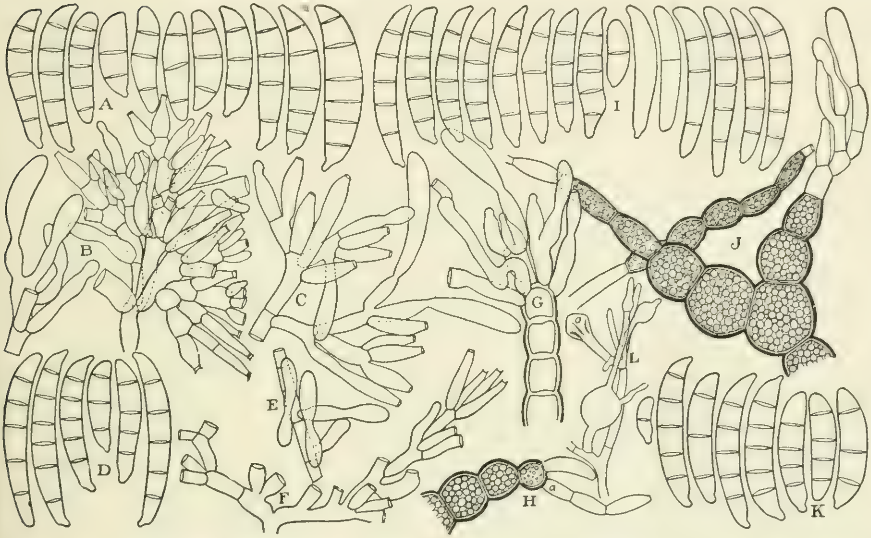

FIG. 43.—*Fusarium culmorum var. leteius.* A, *Conidia,* B, *conidiophores, from aërial sporodochium from 15-days-old culture on slightly acidified hard potato agar;* C, *conidiophore from 11-days-old culture on hard lima-bean agar;* D, *conidia from plectenchymic sporodochium,* E, F, G, *conidiophores, from 72-days-old culture on red raspberry cane plug;* H, *intercalary chlamydospores from 175-days-old culture on corn agar;* I, *conidia from plectenchymic sporodochium from 11-days-old culture on hard lima-bean agar;* J, *intercalary chlamydospores from 175-days-old culture on corn agar (some chlamydospores producing conidiophores);* K, *conidia from aërial sporodochia from 72-days-old culture on red raspberry cane plug;* L, *tip of aërial hypha showing prominent swellings (magnification 250 times)*

On red raspberry cane plug, culture seventy-two days old:

(1) Conidia from a plectenchymic sporodochium borne directly on the plug

Conidia: 3–septate, 14 per cent
4–septate, 20 per cent
5–septate, 66 per cent, 43 x 6.2 (31–53 x 5.8–7)μ

(2) Conidia from a bushlike sporodochium borne on aërial mycelium

Conidia: 1– and 2–septate, very few
3–septate, 40 per cent, 29 x 6.9 (26–35 x 6–7.8)μ
4–septate, 30 per cent, 32 x 6.8 (29–36 x 6–7.5)μ
5–septate,. 30 per cent, 37 x 6.8 (31–42 x 6.3–7.5)μ

On hard lima-bean agar, culture eleven days old; conidia from a sporodochium (only mature spores measured):

Conidia: 1–septate, 2 per cent
3–septate, 26 per cent, 32 x 6.1 (24–37 x 5.2–6.7)μ
4–septate, 37 per cent
5–septate, 35 per cent, 39 x 5.9 (30–44 x 5.3–6.7)μ

Average of the above measurements:

Conidia: 1–septate, 0.5 per cent, 18 x 5.2μ
2–septate, 1.5 per cent, 23.5 x 6.9μ
3–septate, 28 per cent, 27.2 x 6.7μ
4–septate, 31 per cent
5–septate, 39 per cent, 38.85 x 6.6μ
6–septate, exceptional, 44.5 x 8.9μ (only one measured)

XI. SECTION MARTIELLA Wr. (Fig. 1, x_1 to c_2). Wollenweber, H. W., Phytopath. **3**:30, Fig. 1, A to C. 1913

Microconidia on aërial mycelium typically present, from oval to oblong, mostly 0–septate; macroconidia mostly 3–septate, of nearly even diameter throughout or but slightly broader either toward apex or toward base, nearly straight in lower half and more or less curved near apex, with somewhat rounded apex or only slightly pointed or constricted, typically from slightly pedicellate to apedicellate; aërial mycelium mostly from white to a light tint of chamois and drab hues; substratum, on neutral agars, never rose-pink nor pomegranate purple in color, sometimes from vinaceous to blue; color of conidia, except in acid media, mostly from pale buff to drab, often distinctly from green to blue.

53. *Fusarium Martii* Ap. et Wr. (Figs. 1, z_1 and A_2, and 44M)

Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. **8**:78–84, text fig. 5. 1910.

Macroconidia mostly 3–septate, 43.9 x 5.15 (42–46 x 4.9–5.3)μ, and

4–septate, 49.3 x 5.3 (48–50 x 4.9–5.4)μ, often also 5–septate, typically, when mature, of from deep lichen and montpellier green (on corn meal agar) to light olive-drab (on potato agar rich in glucose) and often to dark blue (on potato tuber plug);[56] macroconidia usually produced in abundance in small sporodochia and in pseudopionnotes; chlamydospores terminal and intercalary, single, in clusters, and in short chains, mostly 0–septate, 9.25 x 8.16 (8–11.3 x 7.5–9.3)μ; aërial mycelium typically medium short (from 1 to 4 millimeters), loose, more or less coarsely powdered with conidia, and typically, on potato agar rich in glucose, of from smoke gray to sometimes Chartura drab color; the substratum on the same kind of medium being mostly of from tawny olive to sepia in color.

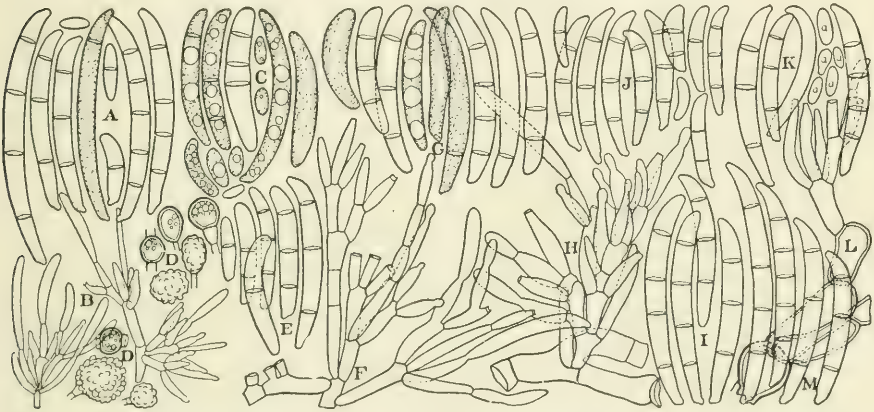

FIG. 44.— A-I, *Fusarium Martii var. viride.* A, *Pseudopionnotal conidia (the stippled one showing dense granulation of the protoplasm masking septation)*, B, *conidiophores (magnification 250 times), from 11-days-old culture on slightly acidified hard potato agar;* C, *conidia showing dense granulation of the protoplasm containing from small to large oil globules from 29-days-old culture on rye straw;* D, *chlamydospores, terminal and intercalary,* E, *pseudopionnotal conidia, from 71-days-old culture on potato tuber plug;* F, *conidiophore from 70-days-old culture on potato stem plug;* G, *conidia,* H, *conidiophore, from 47-days-old culture on stem plug;* I, *pseudopionnotal conidia from 64-days-old culture on potato tuber plug*

J-L, *Fusarium Martii var. minus.* J, *conidia from plectenchymic sporodochium from 71-days-old culture on potato tuber plug;* K, *sporodochial conidia from 85-days-old culture on red raspberry cane plug;* Ka, *microconidia from aërial mycelium;* L, *basal part and one branch of compound conidiophore from 71-days-old culture on potato stem plug*

M, *Fusarium Martii, pseudopionnotal conidia from 11-days-old culture on slightly acidified hard potato agar*

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[56] The culture media are mentioned here merely because the colors were oftener observed on these media than on others; in fact, a green color in conidia is often produced also on potato tuber plugs and on some other media, and the same is true of a blue color. The color is due to color of conidia, not to color of substratum alone, as can be observed under the microscope.

Hab. On rotted tubers of *Solanum tuberosum* and on other plants, in the United States and in Europe. The organism was isolated by the writer from specimens received from various States.

Measurements of conidia on different media are as follows:

On potato tuber plug, culture seventy days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare
1–septate, 3 per cent
2–septate, rare
3–septate, 60 per cent, 46 x 5.3 (38–51 x 5.2–5.7)μ
4–septate, 30 per cent, 50 x 5.4 (43–54 x 5.2–5.9)μ
5–septate, 7 per cent, 52 x 5.6 (45–54 x 5.2–5.9)μ

On slightly acidified hard potato agar, culture eleven days old; conidia from thin pseudopionnotes:

Conidia: 0–septate, 2 per cent, 11 x 4μ
1–septate, 2 per cent, 20 x 4.5μ
2–septate, rare
3–septate, 23 per cent, 43.5 x 5.2 (33–51 x 4.6–5.3)μ
4–septate, 68 per cent, 49 x 5.6 (42–65 x 4.6–6.7)μ
5–septate, 5 per cent, 60 x 5.85 (56–63 x 5.6–6.45)μ

On red raspberry cane plug, culture eighty-two days old; conidia from a sporodochium:

Conidia: 1– and 2–septate, rare
3–septate, 57 per cent, 44 x 5.1 (29–50 x 4.3–5.9)μ
4–septate, 43 per cent, 51 x 5.3 (40–54 x 4.7–5.9)μ
5–septate, rare, 54 x 5.4μ (only one measured)

On potato tuber plug, culture sixty-four days old; conidia from thick pseudopionnotes:

Conidia: 0–septate, rare, about 9 x 3.5μ
3–septate, 55 per cent, 43 x 4.9 (28–50 x 4.7–5.1)μ
4–septate, 45 per cent, 48 x 4.9 (43–55 x 4.7–5.7)μ
5–septate, very rare

On hard lima-bean agar, culture sixty-eight days old; conidia from a small sporodochium:

Conidia: 0– to 2–septate, very rare
3–septate, 48 per cent, 42 x 5.2 (30–51 x 4.8–5.9)μ
4–septate, 50 per cent, 48 x 5.3 (41–53 x 4.8–5.9)μ
5–septate, 2 per cent, 51 x 5.5 (43–56 x 5–5.9)μ

On same medium and of same age as above, conidia from a medium large (about 2 millimeters in diameter), short, column-like sporodochium:

Conidia: 0– to 2–septate, very rare
3–septate, 49 per cent, 45 x 5.2 (36–49 x 4.5–5.6)μ
4–septate, 51 per cent, 50 x 5.2 (38–56 x 4.8–5.6)μ
5–septate, very rare

Average of the above measurements:

Conidia: 0–septate, rare, 11 x 4μ
1–septate, 1 per cent, 20 x 4.5μ
2–septate, very rare
3–septate, 53 per cent, 43.9 x 5.15μ
4–septate, 43 per cent, 49.3 x 5.3μ
5–septate, 3 per cent, 54.3 x 5.57μ

The average sizes given by Appel and Wollenweber for the same fungus are:

Conidia: 3–septate, about 44 per cent, 49 x 5.25μ
4–septate, about 51.5 per cent, 55 x 5.5μ
5–septate, about 4.5 per cent, 56.5 x 5.5μ

54. *Fusarium Martii* Ap. et Wr. var. **viride** n. var. (Fig. 44, A to I; Pl. VI, fig. 5)

Differs from *F. Martii* by having macroconidia somewhat narrower, and usually by a paler color of conidia and substratum; dark blue color of conidial masses not observed. Typical color of conidia in mass, on potato agar rich in glucose, pale smoke-gray, and of substratum pale drab-gray.

Hab. In discolored fibrovascular bundles of *Solanum tuberosum*, in stems and tubers, at Atlanta and Castile, New York.

Measurements of conidia on various media are as follows:

On potato tuber plug, culture seventy-one days old; conidia from pseudopionnotes:

Conidia: 0–septate, 4 per cent
1–septate, 22 per cent
2–septate, 10 per cent
3–septate, 60 per cent, 39 x 5 (33–43 x 4.6–5.2)μ
4–septate, 4 per cent

On slightly acidified hard potato agar, culture eight days old; conidia from pseudopionnotes:

Conidia: 0–septate, rare, 9 x 4μ
1–septate, rare, 17 x 5μ
2–septate, rare
3–septate, 41 per cent, about 46 x 5 (42–51 x 4.4–5.25)μ
4–septate, 58 per cent, 54.4 x 5.25 (45–68 x 4.8–5.6)μ
5–septate, 1 per cent, 58.5 x 5.7 (50–62 x 5–6)μ

On red raspberry cane plug, culture eighty-six days old; conidia from a sporodochium:

Conidia: 3–septate, 50 per cent, 45 x 5.2 (36–53 x 5–5.4)μ
4–septate, 50 per cent, 54 x 5.4 (42–61 x 5.2–5.9)μ

On hard lima-bean agar, culture twenty-two days old; conidia from pseudopionnotes:

Conidia: 3–septate, 90 per cent, 46.1 x 5 (40–49 x 4.7–5.3)μ
4–septate, 9 per cent
5–septate, 1 per cent

On potato tuber plug, culture sixty-four days old; conidia from thick pseudopionnotes:

Conidia: 3–septate, 55 per cent, 45 x 4.9 (40–49 x 4.8–5.3)μ
4–septate, 45 per cent, 49 x 5.3 (45–53 x 5–5.6)μ

On hard lima-bean agar, culture seventy-two days old; conidia from a columnar sporodochial mass:

Conidia: 3–septate, 73 per cent, 45 x 5.1 (40–49 x 4.7–5.3)μ
4–septate, 27 per cent, 50 x 5.2 (47–51 x 5–5.6)μ

Average of the above measurements:

Conidia: 0–septate, 0.5 per cent, 9 x 4μ
1–septate, 3 per cent, 17 x 5μ
2–septate, 1 per cent
3–septate, 63 per cent, 44.4 x 5.03μ
4–septate, 32 per cent, 51.9 x 5.27μ
5–septate, 0.5 per cent, 58.5 x 5.7μ

55. *Fusarium Martii* Ap. et Wr. var. **minus** n. var. (Fig. 44, J to L; Pl. 1, figs. 3 and 4; Pl. VI, fig. 6)

Differs from *F. Martii* and *F. Martii* var. *viride* by having smaller, 3–septate, conidia, 36.7 x 4.8 (30–44 x 4.55–5.1)μ, usually prominent development of plectenchymic, wartlike stromata, and fewer and larger sporodochia (Pl. VI, fig. 6).

Color of substratum, on potato agar rich in glucose, from light gray to drab and dark olive-buff, with a fuscous-colored spot at the point of inoculation (Pl. I, figs. 3 and 4).

Hab. On rotted tubers of *Solanum tuberosum*, evidently following *Phytophthora infestans*, Dutchess County, New York.

Measurements of conidia on different media are as follows:

On potato tuber plug, culture seventy-one days old:

(1) Conidia from a sporodochium

Conidia: 0–septate, 8 per cent
1–septate, 13 per cent
2–septate, 29 per cent
3–septate, 50 per cent, 30 x 4.6 (26–33 x 3.6–4.8)μ

(2) Conidia from pseudopionnotes

Conidia: 1–septate, 9 per cent
2–septate, 37 per cent
3–septate, 54 per cent, 30 x 4.55 (27–34 x 3.9–4.8)μ

(3) Conidia from oldest part of pseudopionnotes

Conidia: 3–septate, 100 per cent, 37 x 4.7 (27–43 x 4.2–5)μ

On slightly acidified hard potato agar, culture eleven days old; conidia from pseudopionnotes:

Conidia: 3–septate, 67 per cent, 43.75 x 4.6 (24–52 x 3.5–5.3)μ
4–septate, 28 per cent, 48.65 x 5 (43–53 x 4.3–5.3)μ
5–septate, 5 per cent, 52.85 x 5 (50–57 x 4.3–5.3)μ

On red raspberry cane plug, culture eighty-five days old; conidia from a sporodochium:

Conidia: 0–septate, rare
1–septate, rare
2–septate, rare
3–septate, 80 per cent, 38 x 5.1 (28–48 x 4.1–5.3)μ
4–septate, 20 per cent, 47 x 5.2 (42–52 x 4.9–5.5)μ
5–septate, rare, 47 x 5.2μ (only one measured)

On hard lima-bean agar with 2 per cent glucose, culture twenty-two days old:

Conidia: 3–septate, 88 per cent, 41.4 x 4.7 (36–47 x 4.1–5.3)μ
4–septate, 10 per cent
5–septate, 2 per cent

On potato tuber plug, culture sixty-four days old; conidia from pseudopionnotes:

Conidia: 0–septate, very rare
3–septate, 86 per cent, 40 x 4.8 (33–49 x 4.1–5.2)μ
4–septate, 14 per cent, 48 x 4.9 (38–56 x 4.7–5.7)μ

On hard lima-bean agar with 2 per cent glucose, culture sixty-eight days old; conidia from a columnar mass:

Conidia: 0– to 2–septate, very rare
3–septate, 93 per cent, 40 x 5.1 (30–45 x 4.7–5.4)μ
4–septate, 7 per cent, 44 x 5.1 (36–48 x 4.7–5.4)μ
5–septate, very rare, 47 x 5.3μ (only one measured)

On same medium, culture also same age; conidia from pseudopionnotes:

Conidia: 0– to 2–septate, very rare
3–septate, 98 per cent, 41 x 5 (33–42 x 4.7–5.3)μ
4–septate, 2 per cent, 44 x 5.1 (40–50 x 4.8–5.3)μ

Average of the above measurements:

Conidia: 0–septate, 11 per cent
1–septate, 2 per cent
2–septate, 6.6 per cent
3–septate, 71.6 per cent, 36.7 x 4.8μ
4–septate, 8.1 per cent, 46.2 x 5.06μ
5–septate, 0.7 per cent, 50 x 5.17μ

56. *Fusarium Solani* (Mart. p. par.) Ap. et Wr. (Fig. 1, X_1 and Y_1; Fig. 45, A to G; Pl. I, fig. 1; Pl. VI, fig. 7)

Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. **8**:64–78; Pl. I, figs. 1 to 30; Pl. III, fig. 1; text fig. 4. 1910.

Microconidia always present, at least on aërial mycelium, same size and shape as those of *F. Martii*. Macroconidia typically somewhat broader

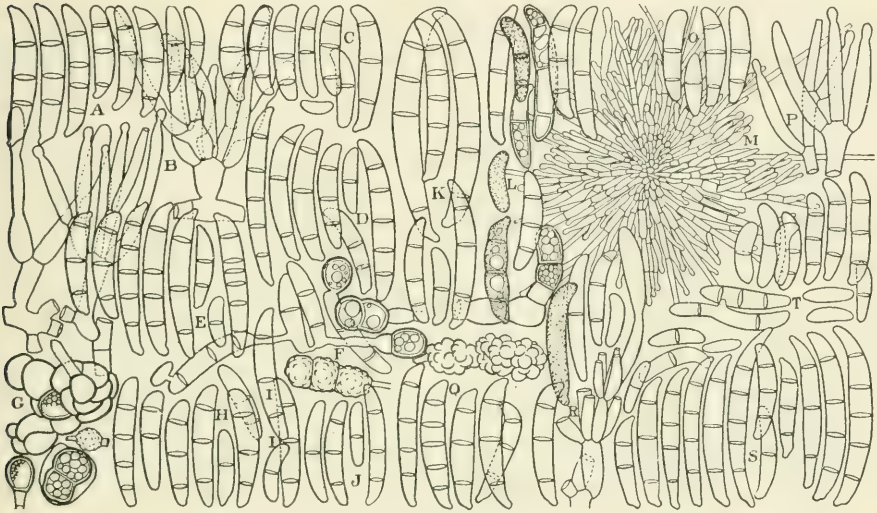

FIG. 45.— A-G, *Fusarium Solani.* A, *Conidia from plectenchymic sporodochia from 66-days-old culture on potato tuber plugs;* B, *conidiophores,* C, *conidia, from aërial mycelium from 12-days-old culture on hard lima-bean agar;* D, *pseudopionnotal conidia from 11-days-old culture on slightly acidified hard potato agar;* E, *conidia from sporodochium from 87-days-old culture on red raspberry cane plug;* F, *terminal, 1- to 3-septate, chlamydospores produced by hyphæ and conidia, the latter anastomosed, from 173-days-old culture on corn agar;* G, *terminal and intercalary, clustered, chlamydospores from 70-days-old culture on potato tuber plug*

H-J, *Fusarium Solani var. cyanum.* H, *Sporodochial conidia from 87-days-old culture on red raspberry cane plug;* I, *pseudopionnotal conidia from 15-days-old culture on slightly acidified hard potato agar;* J, *aërial conidia from 87-days-old culture on red raspberry cane plug*

K-T, *Fusarium Solani var. suffuscum.* K, *Pseudopionnotal conidia from 7-days-old colony in petri dish on hard potato agar (the middle spore above is typical for the culture);* L, *sporodochial conidia from 47-days-old culture on rye straw (many conidia have coarsely granulated protoplasm and oil globules);* M, *compound conidiophore in form of dense tuft (looking from above, magnification 250 times), from 47-days-old culture on potato stem plug;* N, *chlamydospores,* O, *sporodochial conidia, from 70-days-old culture on potato tuber plug;* P, *typical ends of conidiophores from 47-days-old culture on potato stem plug;* Q, *sporodochial conidia from 87-days-old culture on red raspberry cane plug;* R, *sporodochial conidia and conidiophore from 13-days-old culture on potato tuber plug;* S, *sporodochial conidia from 16-days-old culture on slightly acidified hard potato agar;* T, *sporodochial conidia from 47-days-old culture on potato stem plug* in upper half of their length, with from rounded to slightly constricted apex, not at all or slightly pedicellate, typically 3-septate, 29.75 x 5.5 (27–34.7 x 5.4–5.8)μ, sometimes 4-septate, rarely 5 septate; aërial mycelium from poorly to well developed, from white to olive-buff; substratum, on potato agar rich in glucose, olive-buff with a green-blue tinge (Pl. I, fig, 1, and Pl. VI, fig. 7).

Hab. On *Solanum tuberosum* and other substrata, in Europe and America; often in association with other Fusaria.

The organism was often isolated by the writer from rotted potato tubers received from various States.

Measurements of conidia on various media are as follows:

On slightly acidified hard potato agar, culture eleven days old; conidia from pseudopionnotes:

Conidia: 1–septate, 0.5 per cent, 14 x 4 (13–17 x 3.5–5.8)μ
2–septate, 1 per cent, 22 x 4.5 (17–28 x 4.2–4.8)μ
3–septate, 93 per cent, 34.7 x 5.4 (22–42 x 4.3–6.1)μ
4–septate, 5 per cent, 38 x 5.8 (30–43 x 5–6.4)μ
5–septate, 0.5 per cent, 44.3 x 5.8 (38–47 x 5–6.4)μ

On potato tuber plug, culture sixty-five days old; conidia from a sporodochium:

Conidia: 0–septate, rare, 8.7 x 4μ (only a few measured)
1–septate, 2 per cent, 20 x 4.6μ
2–septate, 4 per cent, 23 x 4.7 (18–25 x 4.4–5.4)μ
3–septate, 93 per cent, 28 x 5.8 (22–35 x 4.8–6.1)μ
4–septate, 1 per cent, 33–5.9 (28–38 x 5.4–6.1)μ

On hard lima-bean agar, culture twelve days old:

(1) Conidia from aërial mycelium

Conidia: 0–septate, 20 per cent
1–septate, 40 per cent
2–septate, 19 per cent
3–septate, 21 per cent, 27 x 5.4 (24–37 x 4.7–6)μ

(2) Conidia from a sporodochium

Conidia: 0–septate, 1 per cent
1–septate, 1 per cent
2–septate, 1 per cent
3–septate (97 to 100 per cent), 29.3 x 5.4 (24–35 x 4.8–5.9)μ

Average of the above measurements:

Conidia: 0–septate, 5 per cent, 8.7 x 4μ
1–septate, 11 per cent, 17 x 4.3μ
2–septate, 6 per cent, 22.5 x 4.6μ
3–septate, 76.5 per cent, 29.75 x 5.5μ
4–septate, 1.5 per cent, 35.5 x 5.85μ
5–septate, rare, 44.3 x 5.8μ

57. *Fusarium Solani* (Mart. p. par.) Ap. et Wr. var. **cyanum** n. var. (Fig. 45, H to J)

Differs from *F. Solani* mainly by having macroconidia typically more rounded at apex and base, by fewer 4–septate and absence of 5 septate conidia, and by frequent occurrence of bluish plectenchyma (on hard bean agar and potato tuber plugs); sometimes distinct blue color in conidia also observable; size for the same septation type of conidia almost the same as that of *F. Solani*.

Hab. Same as for *F. Solani*, but much less common.

On potato tuber plug, culture sixty-eight days old; conidia from a sporodochium:

Conidia: 0–septate, 28 per cent
1–septate, 22 per cent
2–septate, 35 per cent
3–septate, 15 per cent, 28 x 5.2 (24–31 x 4.8–5.7)μ

On slightly acidified hard potato agar, culture fifteen days old; conidia from pseudopionnotes:

Conidia: 0–septate, 14 per cent, 12 x 3.7 (8.7–14 x 2.5–5)μ
1–septate, 22 per cent, 24.5 x 4.5 (15–30 x 3.5–5.3)μ
2–septate, 19 per cent, 29 x 5 (24–32 x 4.8–5.3)μ
3–septate, 45 per cent, 33 x 5.7 (31–35 x 5–6)μ

On red raspberry cane plug, culture eighty-seven days old; conidia from a sporodochium:

Conidia: 0–septate, few
1–septate, 3 per cent
2–septate, 5 per cent, 25 x 5.5 (19–27 x 4.7–5.8)μ
3–septate, 90 per cent, 30 x 5.7 (28–35 x 5.2–5.9)μ
4–septate, 2 per cent, 35 x 5.5 (33–37 x 5.2–5.7)μ

On potato tuber plug, culture sixty-five days old; conidia from a sporodochium:

Conidia: 0–septate, few
1–septate, 12 per cent, about 22 x 5μ (only one measured)
2–septate, 16 per cent, about 27 x 5.3μ (only one measured)
3–septate, 72 per cent, 29 x 5.6 (24–35 x 4.8–5.9)μ

On corn agar, culture one hundred and seventy-five days old; conidia only few, chlamydospores numerous, terminal and intercalary:

Chlamydospores: 0–septate, in mycelium, 11.3 x 9.3 (8.5–16 x 7.5–11)μ
1–septate, in mycelium, 16.3 x 9.9 (14–20 x 8–14)μ

On hard lima-bean agar, culture twelve days old; conidia from pseudopionnotes:

Conidia: 0–septate, 4 per cent } (percentage may vary considerably
1–septate, 15 per cent } from somewhat greater than figures
2–septate, 16 per cent } given here to nearly zero)
3–septate, 65 per cent, 33 x 5.2 (27–37 x 4.7–5.6)μ

Average of the above measurements:

Conidia: 0–septate, 9 per cent, 12 x 3.7μ
1–septate, 15 per cent, 23 x 4.75μ
2–septate, 18 per cent, 27 x 5.3μ
3–septate, 57.5 per cent, 30.5 x 5.5μ
4–septate, 0.5 per cent, 35 x 5.5μ

58. *Fusarium Solani* (Mart. p. par.) Ap. et Wr. var. **suffuscum** n. var. (Fig. 45, K to T)

Differs from *F. Solani* and *F. Solani* var. *cyanum* mainly by typically well-developed, uniform, fine, aërial mycelium, with mass of chlamydospores at maturity which gives it a pale brownish tint; by sparse conidial production on aërial mycelium; and by the fact that sporodochia are usually few and distant from one another.

Hab. On rotted tubers of *Solanum tuberosum*, Wisconsin.

The average size and occurrence of septation type of conidia on various media are as follows:

Conidia: 0–septate, 5.5 per cent, 10.7 x 3.15μ
1–septate, 13.5 per cent, 20 x 4.5μ
2–septate, 17 per cent, 25 x 4.5μ
3–septate, 60.5 per cent, 30 x 5.41μ
4–septate, 3.5 per cent, 34.5 x 5.5μ
5–septate, rare, 42.25 x 5.85μ

59. **Fusarium striatum** n. sp. (Figs. $1c_2$ and 46; Pl. I, fig. 2)

Microconidia, at least on aërial mycelium, always present. Macroconidia of shape and septation intermediate between *F. Martii* and *F. Solani*, mostly 3–septate, 34.7 x 4.6 (31–36 x 4.4–5)μ, from colorless to yellowish glaucous and pale turquoise green, in numerous minute sporodochia; sporodochia often converging into a pseudopionnotes; aërial mycelium short (rarely up to 3 millimeters high), typically (on various agars) fine, uniformly from loose to very loose, downy in appearance, from white to grayish white; substratum, on agars rich in glucose, from pale glaucous green to tawny olive and sepia.

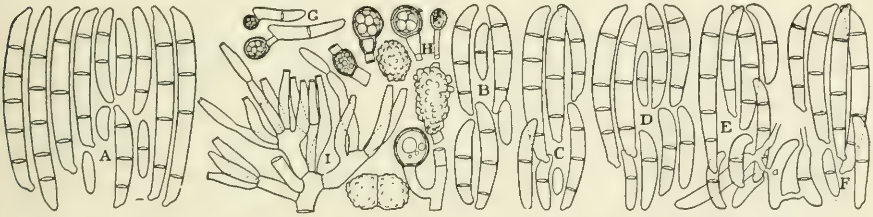

Fig. 46.—*Fusarium striatum.* A, *Pseudopionnotal conidia from 17-days-old culture on slightly acidified hard potato agar;* B, *pseudopionnotal conidia from 58-days-old culture on oats;* C, *conidia from small sporodochium from 32-days-old culture on hard lima-bean agar;* D, *sporodochial conidia from 86-days-old culture on red raspberry cane plug;* E, *pseudopionnotal conidia from 13-days-old potato tuber plug (some of them anastomosing);* F, *pseudopionnotal conidia from 12-days-old culture on hard lima-bean agar;* G, *terminal chlamydospores produced by conidia from 64-days-old culture on hard lima-bean agar with 2 per cent glucose;* H, *chlamydospores from 32-days-old culture on hard lima-bean agar;* I, *compound conidiophore from 34-days-old culture on hard lima-bean agar*

Hab. On tubers of *Solanum tuberosum*, Colorado.

Latin description.—Microconidiis — saltem in aerio mycelio — semper praesentibus; macroconidiis forma et septatione inter F. Martii et F.

Solani mediis, plerumque 3–septatis, 34.7 x 4.6 (31–36 x 4.4–5)μ, ex hyalino luteolo-glaucis vel pallide "turquoise green" (R), multis minutis sporodochiis; sporodochiis saepe in pseudopionnotem vergentibus; aerio mycelio brevi (usque ad 3 mm. alt.) typice (in agaribus variis) subtili, aequabiliter laxo vel laxissimo, pubescenti viso, ex albo caesio-albo; substrato — in agaribus perglucosis — ex pallide glauco-viridi "tawny-olive" (R) et "sepia" (R).

Hab. In tuberibus Solani tuberosi, Colorado, Amer. bor.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture seventeen days old; conidia from pseudopionnotes:

Conidia: 0–septate, 6 per cent, 10.6 x 3.15 (8.5–12.5 x 2.5–3.5)μ
1–septate, 10 per cent, 19.5 x 3.9 (14–21 x 3–4.4)μ
2–septate, 4 per cent, 24.15 x 4.2 (21–27 x 3.5–4.7)μ
3–septate, 64 per cent, 36.4 x 4.4 (26–50 x 4–5)μ
4–septate, 16 per cent, 47 x 4.5 (40–56 x 4.3–5.3)μ
5–septate, rare, 50 x 4.8 (47–56 x 4.3–5.3)μ

On red raspberry cane plug, culture eighty-six days old; conidia from a sporodochium:

Conidia: 0–septate, 3 per cent
1–septate, 7 per cent, 19 x 3.5 (12–23 x 3–4.1)μ
2–septate, 2 per cent
3–septate, 88 per cent, 41.6 x 5 (24–44 x 4–5.7)μ
4–septate, very rare, 43 x 5 (only one measured)

On hard lima-bean agar with 2 per cent glucose, culture sixty-four days old; conidia from pseudopionnotes:

Conidia: 0–septate, 10 per cent
1–septate, 22 per cent, 22 x 4.1 (16-30 x 3.5–4.7)μ
2–septate, 10 per cent
3–septate, 58 per cent, 31 x 4.6 (22–37 x 4.2–5)μ

On rye grain, culture sixty-four days old; conidia from a sporodochium:

Conidia: 0–septate, 39 per cent
1–septate, 28 per cent, 20 x 3.9 (14–23 x 3.5–4.4)μ
2–septate, 5 per cent
3–septate, 28 per cent, 31 x 4.6 (24–36 x 4.3–5)μ

On hard lima-bean agar, culture twelve days old:

(1) Conidia from a sporodochium

- 0–septate, 13 per cent
- 1–septate, 30 per cent
- 2–septate, 7 per cent
- 3–septate, 50 per cent, 33 x 4.4 (24–44 x 3.6–4.7)μ
- 4–septate, very few

(2) Chlamydospores, terminal and intercalary, mostly 0–septate, 9 x 7.5 (7–11 x 6–9)μ

On same medium as above, culture thirty-two days old; conidia from a sporodochium:

Conidia: 0–septate, 1 per cent, 9 x 3.6μ (only a few measured)
1–septate, 20 per cent, 20 x 3.9 (14–25 x 3.5–4.1)μ
2–septate, 5 per cent, 25 x 4.2 (23–30 x 4–4.4)μ
3–septate, 73 per cent, 35 x 4.6 (28–37 x 4–4.7)μ
4–septate, 1 per cent, 41 x 4.7μ (only a few measured)

Average of the above measurements:

Conidia: 0–septate, 12 per cent, 10 x 3.4μ
1–septate, 19.5 per cent, 20 x 3.9μ
2–septate, 5.5 per cent, 24.5 x 4.2μ
3–septate, 60 per cent, 34.7 x 4.6μ
4–septate, 3 per cent, 43.7 x 4.7μ
5–septate, very rare, 50 x 4.8μ

60. *Fusarium radicicola*[57] Wr. (Fig. 47; Pl. VI, fig. 8)

Wollenweber, H. W., Journ. Agr. Research **2**:257-258, Pl. XVI, fig. K, 1914.

Microconidia nearly straight near base, slightly curved in upper third of their length, with from somewhat rounded to distinctly constricted apex, slightly pedicellate, mostly 3–septate, 35.2 x 4.7 (31–40 x 4.6–5)μ; 0– and 1–septate, microconidia very common, 0–septate measuring 8 x 3μ; chlamydospores common, terminal and intercalary, mostly 0– and 1–septate, 0–septate averaging 9–10 x 8.7–8.8μ; pseudopionnotes typically absent; plectenchymic sporodochia often present; aërial mycelium well developed; color of conidia from white to olive, of substratum from pale yellowish to olive (on agar rich in glucose and on potato tuber plug).

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[57] In an article which appeared after this work was ready for press, Dr. Wollenweber (1914) describes a new species of Fusarium (*F. radicicola*) with which this organism appears identical. No cultural comparisons have been possible, but the type of tuber rot and type of conidia are alike, and in certain instances material for study has come from the same region. Possibly any differences may be accounted for in the difference in strains studied.

Hab. On rotted tubers of *Solanum tuberosum*, in Oregon, Idaho, and California.

F. radicicola differs from *F. Martii* and *F. Martii* var. *viride* by shorter macroconidia; from *F. Martii* var. *minus* by absence of prominent plectenchymic sporodochia; and from *F. striatum* by well-developed aërial mycelium and by typical absence of pseudopionnotes.

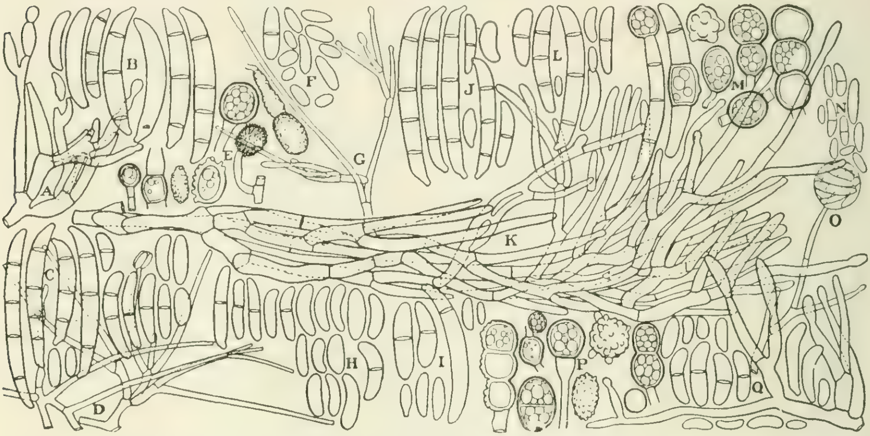

FIG. 47.— *Fusarium radicicola.* A, *Conidiophore,* B, *sporodochial conidia, from 67-days-old culture on potato stem plug;* C, *aërial and pseudopionnotal conidia from 12-days-old culture on hard lima-bean agar;* D, *conidiophore (magnification 250 times) from 48-days-old culture on rye straw;* E, *chlamydospores,* F, *aërial conidia,* G, *conidiophore (magnified 250 times), from 73-days-old culture on red raspberry cane plug;* H, *conidia from the surface of original stem-end rotted potato tuber;* I, *conidia from 48-days-old culture on rye straw;* J, *conidia from pseudopionnotes in 7-days-old colony in petri dish on hard potato agar;* K, *conidiophore from the surface of original stem-end rotted potato tuber;* L, *aërial conidia close to substratum from 64-days-old culture on hard lima-bean agar;* M, *chlamydospores produced in mycelium and conidia from 173-days-old culture on corn agar;* N, *conidia,* O, *spore ball, both magnified 250 times, from 3-days-old hanging drop culture in potato decoction in van Tieghem cell;* P, *chlamydospores from 82-days-old culture on potato tuber plug;* Q, *conidia and conidiophores from 26-days-old culture on oats*

Production of macroconidia in sporodochia was observed to be abundant only under certain conditions (not well determined as yet), but usually microconidia alone are the dominant type. The Oregon specimens, from which cultures of the fungus were first obtained, showed a dark, depressed area of dry rot at the stem end of the tubers, with cream-white, dense tufts of conidiophores, up to 1 centimeter high and powdered with conidia. (For microscopic characters see figure 47.) Pure cultures of this organism were obtained both from the aërial conidia and from plantings of the rotted tissues of the tubers.

The organisms in general differ much from *F. striatum*, but in certain cultures (in sporodochia-producing stage on whole steamed potato tubers) resemble it very closely.

Measurements of conidia on different media are as follows:

On potato tuber plug, culture eighty-two days old:
(1) Conidia from aërial mycelium
Conidia: 0–septate, 95 per cent, 8 x 3 (6–15 x 2.1–4)μ
1–septate, 5 per cent, 15 x 4 (13–21 x 3.4–4.4)μ
3–septate, rare
(2) Chlamydospores, intercalary and terminal, unicellular and in chains
0–septate, 9.8 x 8.7 (5.2–16 x 5.2–12)μ

On hard lima-bean agar with 2 per cent glucose, culture sixty-four days old:
(1) Conidia from aërial mycelium close to substratum
Conidia: 0–septate, 80 per cent
1–septate, 17 per cent
2–septate, 2 per cent
(size same as above)
3–septate, 1 per cent, 33 x 4.8 (22–41 x 4.3–5.2)μ
4–septate, rare, 45 x 4.8 (only a few measured)
(2) Chlamydospores
0–septate, 9.5 x 8.8 (6.1–12 x 5.2–11)μ
1–septate, 18 x 10 (16–22 x 5–12)μ

On hard lima-bean agar, culture twelve days old:
(1) Conidia from aërial mycelium
Conidia: 0–septate, 60 per cent
1–septate, 30 per cent
2–septate, 1 per cent
3–septate, 6 per cent, 37 x 4.5 (28–48 x 4–5.3)μ
4–septate, 3 per cent, 43.6 x 5 (40–49 x 4.3–5.2)μ
5–septate, rare, size about that of 4–septate
(2) Chlamydospores, mostly 0–septate, 9 x 8.7μ

On whole steamed potato tuber, culture sixty days old; conidia from a sporodochium:

Conidia: 0–septate, 11 per cent
1–septate, 21 per cent
2–septate, 8 per cent
3–septate, 60 per cent, 31 x 4.8 (29–38 x 4.2–5.5)μ
4–septate, very rare

On oat grain, culture fifty-seven days old; conidia from a sporodochium, septation as above:

Conidia: 3–septate, 35.2 x 4.7 (25–42.5 x 4.2–5)μ
4–septate, 43 x 5μ (only one measured)

On potato stem plug, culture seventy days old; conidia from a sporodochium, septation approximately that given above:

Conidia: 3–septate, 35.2 x 5 (24.5–38 x 3.7–5.5)μ
4–septate, 41.6 x 5.8μ (only one measured)

On hard potato agar, culture ten days old; conidia from aërial mycelium close to substratum:

Conidia: 0–septate, 30 per cent
1–septate, 10 per cent
2–septate, 5 per cent
3–septate, 50 per cent, 40 x 4.6 (32–46 x 4.3–4.9)μ
4–septate, 5 per cent, 47 x 4.8 (45–49 x 4.5–5)μ

Average of the above measurements:

Conidia: 0–septate, 56 per cent (from 11 to 95 per cent), 8 x 3μ
1–septate, 16 per cent (from 5 to 30 per cent), 15 x 4μ
2–septate, 3 per cent (from 0 to 8 per cent)
3–septate, 23 per cent (from 0 to 60 per cent), 35.2 x 4.73μ
4–septate, 2 per cent (from 0 to 5 per cent), 43.9 x 5.1μ
5–septate, very rare, size about that of 4–septate

61. *Fusarium coeruleum* (Lib.) Sacc. (Figs. 1B$_2$ and 48; Pl. I, figs. 5 and 6; Pl. VI, fig. 4)

Syn. *Fusarium violaceum* Fuckel. 1869.
Selenosporium coeruleum Libert, in herbarium.

Cf. Saccardo, Syll. Fung. **4**:705. 1886. Appel, O., and Wollenweber, H. W., Arb. K. biol. Anst. Land- u. Forstw. **8**:84–91, Pl. III fig. 6, text fig. 6. 1910. Wollenweber, H. W., Phytopath. 3:31, 44, 45, Fig. 1c. 1913.

Microconidia of larger size than those of other species of section Martiella, 0–septate, about 16 x 4.7μ. Macroconidia, for the largest part, of an even diameter or somewhat broader toward the base, only slightly curved near, and more or less rounded at, the apex, never apically constricted, mostly apedicellate or with ventrally depressed basal cell, mostly 3–septate, 33.3 x 5 (30–36 x 4.5–5.4)μ; aërial mycelium usually medium well developed, feltlike in age, of from white, bluish white, and olive-buff to dusky slate violet, on potato agar rich in glucose, and to slate purple on corn meal agar; substratum, on potato agar rich in glucose, from deep hyssop violet to indian lake, ocher-red, and, in older cultures, violet-carmine; color of conidia from orchraceous orange, on strong acid agars, to pale buff and mouse gray, or often blue, on neutral media.

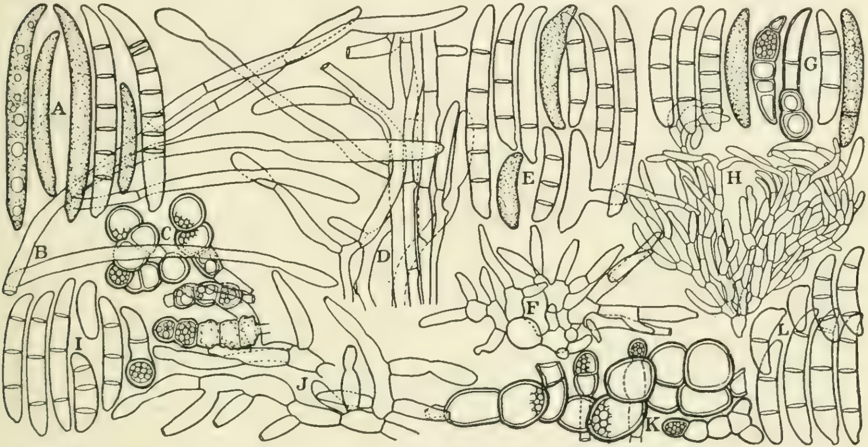

FIG. 48.—*Fusarium coeruleum.* A, *Pseudopionnotal conidia,* B, *conidiophore, from 8-days-old colony in petri dish on hard potato agar;* C, *chlamydospores,* D, *coremium-like form of mycelial growth with conidiophores,* E, *pseudopionnotal conidia, from 35-days-old culture on potato tuber plug;* F, *conidiophore from 62-days-old culture on rye straw;* G, *conidia (some with chlamydospores),* H, *conidiophore (magnified 250 times), from 54-days-old culture on rye straw;* I, *conidia from small sporodochium,* J, *conidiophore, from 79-days-old culture on red raspberry cane plug;* K, *chlamydospores,* L, *pseudopionnotal conidia, from 22-days-old culture on hard potato agar.* A, B, F, G, *and* H *are from strains isolated from rotted potato tubers received from American sources, the remainder are from the culture received from Dr. Wollenweber*

Hab. On rotted tubers of *Solanum tuberosum,* common in Europe and

America. Cause of most of Fusarial rot on potatoes in storage, often occurring in association with other Fusaria.

The organism was repeatedly isolated by the writer from diseased tubers, and every strain isolated proved capable of producing more or less dark-colored dry rot on tubers. It may be mentioned here that a white rot of tubers can be produced by certain species of Fusarium of the Elegans section, such as *F. lutulatum.* A rot distinctly striate in appearance was often produced by inoculation with *F. striatum*; a brownish rot of tubers is caused by *F. trichothecioides*; and a more or less pinkish rot with large cavities, more or less covered with red masses of macroconidia, is produced by *F. subulatum* and *F. subulatum* var. *brevius.*

Of all these, *F. coeruleum* is the commonest and most vigorous wound parasite of potato tubers; the next, perhaps, is *F. subulatum* and its variety.

Measurements of conidia of a few strains of the species on different media are as follows:

On slightly acidified hard potato agar, culture twenty-two days old; conidia from pseudopionnotes, strain 66:

Conidia: 0–septate, 5 per cent, 16 x 4.7 (6–28 x 4.3–5.5)μ
1–septate, 9 per cent, 21 x 4.7 (17–27 x 4.4–5.3)μ
2–septate, 10 per cent, 25 x 5.3 (18–32 x 4.6–5.9)μ
3–septate, 75 per cent, 30 x 5.4 (20–40 x 4.7–6.1)μ
4–septate, 1 per cent, 36 x 5.5 (33–40 x 5.2–6.1)μ

On red raspberry cane plug, culture seventy-nine days old; conidia from a sporodochium, strain 66; 3–septate, up to 100 per cent, 33 x 5.4 (22–40 x 4.8–6)μ; average septation being

Conidia: 0–septate, 3 per cent
1–septate, 11 per cent
2–septate, 8 per cent
3–septate, 78 per cent
4–septate, rare

On corn meal agar, culture one hundred and seventy-five days old; strain 53; very few conidia observed; chlamydospores numerous:

Chlamydospores: 0–septate, 8 x 7.5 (5.2–10.5 x 5.2–9)μ
1–septate, 11.7 x 7.7 (9.2–14 x 7–8.5)μ
Also in chains in mycelium, 9 (7.8–11)μ in diameter

On hard potato agar, culture one hundred and fifty days old; conidia from a sporodochium, strain 96:

Conidia: 0–septate, 10 per cent
1–septate, 12 per cent
2–septate, 7 per cent
3–septate, 71 per cent, 36 x 5.1 (33–40 x 4.4–5.5)μ
Chlamydospores: 0–septate, in conidia, average size 8.8 x 8μ

On potato tuber plug, culture eighty-five days old; conidia from a pseudopionnotes, strain 66:

Conidia: 0–septate, 5 per cent
1–septate, 8 per cent
2–septate, 4 per cent
3–septate, 68 per cent, 34.2 x 4.5μ
4–septate, 9 per cent, 42 x 4.6μ (only one measured)
5–septate, 6 per cent, 55 x 5.5μ (only two measured)

On rye straw, culture fifty-four days old; conidia from a sporodochium, strain 96:

Conidia: 0–septate, 2 per cent
1– to 2–septate, 5 per cent
3–septate, 89 per cent, 33.5 x 5.1 (30–37 x 4–6)μ
4–septate, 4 per cent, 33 x 6.1μ (only one measured)

On rye straw, culture fifty days old; conidia from a sporodochium, strain 66:

Conidia: 0– to 2–septate, 5 per cent
3–septate, 88 per cent, 30.5 x 4.9 (28–33 x 4.5–5.3)μ (only three measured)
4–septate, 7 per cent, 33.2 x 5.1μ (only one measured)

On rye straw, culture forty-three days old; conidia from a sporodochium, strain 53:

Conidia: 0– to 2–septate, 23 per cent
3–septate, 73 per cent, 35 x 5 (25–46.2 x 4.7–5.3)μ
4–septate, 4 per cent, 50 x 5μ (only one measured)

On potato hard agar, culture eight days old; strain 190:

Conidia: 0– to 2–septate, 2 per cent
3–septate, 80 per cent
4– and 5–septate, 10 per cent
6– and 7–septate, 8 per cent, 58 x 5.6 (57–60 x 5.2–6)μ

The greatest septation observed was in a culture fifteen days old on oat grains. This was a 9–septate conidium measuring 39 x 5.2μ.

Average of the above measurements:

Conidia: 0–septate, about 4 per cent, 16 x 4.7μ
1–septate, about 6 per cent, 21 x 4.7μ
2–septate, about 5 per cent, 25 x 5.3μ
3–septate, about 78 per cent, 33.3 x 5μ
4–septate, about 4.5 per cent, 39 x 5.26μ
5–septate, about 2 per cent, 55 x 5.5μ
6– and 7–septate, about 0.5 per cent, 58 x 5.6μ

Appel and Wollenweber's measurements for 3–septate conidia average 36 x 5.25 (31–40 x 4.5–5.5)μ

RAMULARIA (Unger) Fries (Fig. 1, A to C)

Unger, F., Exantheme der Pflanzen, page 169. 1833. Fries, E. M., Summa vegetabilium Scandinaviae, page 493. 1849. Cf. Wollenweber, H. W., Phytopath. 3:33. 1913. Wollenweber, H. W., Phytopath. 3:207–211. 1913.

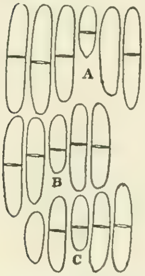

Fig. 49.—*Ramularia eudidyma.* A, *Conidia from aërial mycelium from 55-days-old culture on red raspberry cane plug;* B, *sporodochial conidia from 153-days-old culture on hard lima-bean agar;* C, *conidia from aërial mycelium from 25-days-old culture on slightly acidified hard potato agar*

Differs from Fusarium mainly by nearly cylindrical, apedicellate conidia, with rounded apex; plectenchymic stromata flat to well-developed, wartlike, short column-like structures typically present; conidia borne on conidiophores on aërial mycelium, or on plectenchymic substratum; microconidia (that is, a distinct, abbreviated type of conidia) absent.

1. *Ramularia eudidyma* Wr. (Figs. 1B and 49)

Wollenweber, H. W., Phytopath. 3:221–222, Pl. XXI, fig. C. 1913.

Syn. *Fusisporium didymum* Hartig. 1846.
Fusarium didymum (Hart.) Lindau. 1909.
Fusarium didymum (Hart.) Ap. et Wr. 1910.
Ramularia didyma (Hart.) Wr. 1913.

Conidia nearly cylindrical, with both ends rounded or basal ends sometimes papillate, mostly 1–septate, 23 x 4.87 (21–26 x 4.7–5)μ;[58] 0– to 2–septate conidia also found, 3–septate very rare; chlamydospores mostly intercalary, 8–11μ in diameter; color of spore mass, from white to yellowish; color of plectenchyma, dense brown.

---

[58] According to Wollenweber's data (1913 c: 234) the average size of 1–septate conidia is 26.4 x 5 (21–29 x 4.25–5.5)μ.

Hab. On decaying tubers of *Solanum tuberosum* and on roots of *Rubus idœus*, also in soil, Europe.

The organism was not isolated by the writer, but was briefly studied from a culture received through the courtesy of Dr. Wollenweber.

2. *Ramularia Magnusiana* (Sacc.) Lindau (Figs. 1c and 50)

Cf. Wollenweber, H. W., Phytopath. 3:221, 234; Pls. XX, figs. F to H, and XXI, fig. A. 1913.

Conidia of the same type as those of *R. eudidyma*, also mostly 1–septate, 23.6 x 4.3 (18–27 x 3.5–5)μ;[59] 0– to 3–septate conidia also occurring, not numerous, sometimes catenulate; plectenchyma from olive to coffee brown. Much like *R. candida* (Ehr.) Wr., differing from it by old rose color on rice and by thinner conidia sometimes borne in chains. 0–septate chlamydospores 10 x 11 (7–11 x 9–16)μ; 1–, 2–, and pluri–septate chlamydospores also occurring.

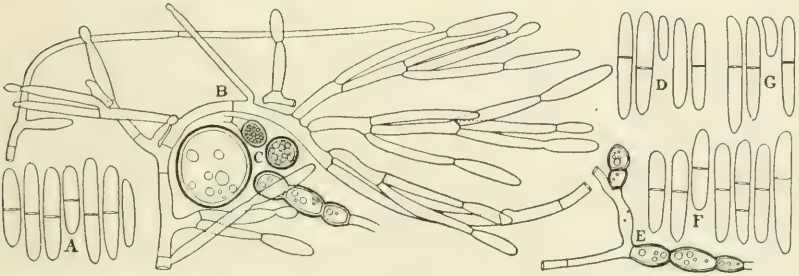

FIG. 50.— *Ramularia Magnusiana.* A, *Conidia from aërial mycelium,* B, *conidiophores, from 11-days-old culture in a petri dish on neutral hard potato agar;* C, *chlamydospores,* D, *sporodochial conidia, from 51-days-old culture on red raspbery cane plug;* E, *chlamydospores from 11-days-old culture in a petri dish on neutral hard potato agar;* F, *conidia from aërial mycelium from 22-days-old culture on slightly acidified hard potato agar;* G, *sporodochial conidia from 153-days-old culture on hard lima-bean agar with 2 per cent glucose*

Hab. Common on tubers of *Solanum tuberosum*, also found on the leaves of Trientalis and on the roots of Acer, in Europe and America.

The fungus was isolated several times from rotted potato tubers, New York, the tubers being covered with from a few to many, from dark brown to brick red, plectenchymic bodies, from 2 to 3 millimeters in diameter and from 1 to 4 millimeters high, often bearing characteristic, almost cylindrical, usually 1–septate, conidia.

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[59] The size is after Wollenweber (1913 c: 234).

The strains isolated by the writer differ from those described by Wollenweber, by more reddish color of plectenchyma, by somewhat narrower conidia and larger (?)[60] chlamydospores, and by the absence of persistent conidial chains.

Measurements of conidia of strain 63 on a few different media are as follows:

On slightly acidified hard potato agar, culture twenty-two days old; conidia from aërial mycelium:

Conidia: 0–septate, rare
1–septate, 95 per cent, 26.5 x 4.1 (18–30 x 3.5–4.7)µ
2–septate, 5 per cent, about the same size as 1–septate

On red raspberry cane plug, culture fifty-one days old; conidia from a sporodochium:

Conidia: 0–septate, 50 per cent, 13 x 3 (8–28 x 2.6–4)µ
1–septate, 50 per cent, 27 x 3.9 (24–31 x 3–4.3)µ

On hard lima-bean agar, culture one hundred and fifty-three days old; conidia from a sporodochium:

Conidia: 0–septate, 1 per cent, 10 x 3.4 (7.9–12.3 x 3–3.5)µ
1–septate, 99 per cent, 26 x 3.8 (20–32 x 3.5–4)µ

Average of the above measurements:

Conidia: 0–septate, 17 per cent, 11.5 x 3.9µ
1–septate, 81 per cent, 26.5 x 3.9µ
2–septate, 0 to 5 per cent, same as 1–septate

The average size of conidia according to Wollenweber's measurements is:

Conidia: 0–septate, rare, 14 x 3.8µ
1–septate, 100 per cent, 23.6 x 4.3µ
3–septate, rare, 30 x 5µ

It is possible that strain 63, on a thorough comparative study of it with *R. Magnusiana*, may prove to be a distinct variety; but it is evident that the resemblance between the two in all important characters is close enough to warrant its being placed in the species *R. Magnusiana*.

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[60] Wollenweber does not give the size of chlamydospores, but it can be interpreted that it is the same as for *R. candida* (Ehr.) Wr., namely, 5–8µ in diameter. Chlamydospores of the strains described here on corn meal agar in cultures one hundred and seventy-three days old measured 10 x 11 (7–11 x 9–16)µ.

3. **Ramularia Solani** n. sp. (Figs. 1A and 51)

Conidia from nearly cylindrical to slightly curved, with both ends rounded or with basal cell slightly papillate, mostly 1- and 2-septate; 1-septate measuring 29.5 x 6.5 (28–30 x 6.2–6.7)μ, and 2-septate measuring 32 x 6.4 (26–33.7 x 6.3–6.8)μ; plectenchyma flat, usually chocolate brown; aërial mycelium from 2 to 4 millimeters high, from medium dense to medium loose, from chamois to deep olive-buff, on potato agar rich in glucose, often with a chocolate-drab tinge; substratum, on the same medium, in zones of from sepia to tawny olive, and in old cultures to Saccardo's amber; conidia in mass from nearly white to deep olive-buff; no chlamydospores observed.

Hab. On rotted tubers of *Solanum tuberosum*, New York.

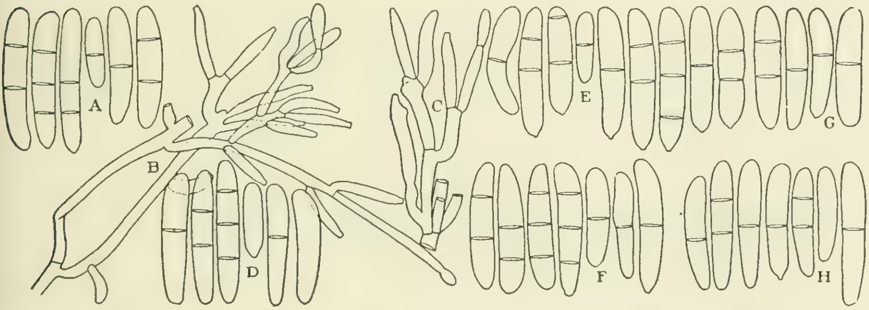

FIG. 51.— *Ramularia Solani.* A, *Sporodochial conidia from 12-days-old culture on oats;* B *and* C, *conidiophores from 34-days-old culture on hard lima-bean agar;* D, *conidia from aërial mycelium from 25-days-old culture on slightly acidified hard potato agar;* E, *conidia from aërial mycelium from 34-days-old culture on hard lima-bean agar;* F, *sporodochial conidia from 8-days-old culture on hard lima-bean agar;* G, *sporodochial conidia from 55-days-old red raspberry cane plug;* H, *sporodochial conidia from 67-days-old flask culture on hard potato agar with 2 per cent glucose*

Latin description.—Conidiis ferme cylindricis vel parum curvatis, utrisque finibus rotundatis vel cella infima parum papillata, plerumque 1–2–septatis; conidiis 1–septatis, 29.5 x 6.5 (28–30 x 6.2–6.7)μ, vel 2–septatis, 32 x 6.4 (26–33.7 x 6.3–6.8)μ; plectenchymate plano, plerumque "chocolate brown" (R); aerio mycelio 2–4 mm. alt., e mediocriter denso mediocriter laxo, e "chamois" (R) "olive-buff" (R), saepe in agare Solani tuberosi perglucoso "chocolate-drab" (R) tincto; substrato, eodem in agare, e "sepia" (R) "tawny olive" (R) in zonis, vel in culturis maturis

"Saccardo's amber" (R); conidiis in totum e ferme albo olivaceo-gilvis; nullis chlamydosporis.

Hab. In tuberibus putridis Solani tuberosi, New York, Amer. bor.

Measurements of conidia on different media are as follows:

On slightly acidified hard potato agar, culture twenty-five days old; conidia from a sporodochium:

Conidia: 0–septate, rare
1–septate, 25 per cent, 28–35 x 5.5–6.3μ
2–septate, 45 per cent, 26 x 6.3 (29–39 x 6.7)μ
3–septate, 30 per cent, 37 x 6.5 (30–41 x 6.3–7)μ

On red raspberry cane plug, culture fifty-five days old; conidia from a sporodochium:

Conidia: 1–septate, 63 per cent, 30 x 6.7 (20–34 x 5.5–7.4)μ
2–septate, 37 per cent, 33 x 6.8 (25–37 x 6.5–7.4)μ

On the same medium as above, culture one hundred and thirty-six days old; conidia from a sporodochium:

Conidia: 0–septate, few, 19 x 5.4μ (only a few measured)
1–septate, 30 per cent, 28 x 6.2 (20–32 x 5.2–6.8)μ
2–septate, 62 per cent, 33 x 6.5 (28–40 x 6–7)μ
3–septate, 8 per cent, 35 x 6.5 (31–40 x 6–7)μ

On hard lima-bean agar, culture eight days old; conidia from a sporodochium:

Conidia: 1–septate, 44 per cent, 30 x 6.3 (22–35 x 5.5–6.5)μ
2–septate, 49 per cent, 33.7 x 6.4 (29–39 x 6–6.6)μ
3–septate, 7 per cent, 36 x 6.5 (31–40 x 6–6.6)μ

On same medium and from same sporodochium as above, culture thirty-four days old:

Conidia: 0–septate, rare
1–septate, 46 per cent, 30 x 6.2 (23–37 x 5.4–6.5)μ
2–septate, 35 per cent, 33.6 x 6.3 (24–39 x 5.8–6.5)μ
3–septate, 19 per cent, 36.9 x 6.5 (32–39 x 6.1–7)μ

Average of the above measurements:

Conidia: 0–septate, rare, 19 x 5.4μ
1–septate, 42 per cent, 29.5 x 6.5μ
2–septate, 45 per cent, 32 x 6.4μ
3–septate, 13 per cent, 36 x 6.5μ

This organism, together with *Fusarium udum* var. *Solani*, was isolated by the writer only once, from a planting of diseased tissues of a potato tuber affected with a superficial dry rot, received from Long Island, New York.

By the shape and size of its conidia it very closely resembles *R. macrospora* Fres. (see Wollenweber 1913 c:222–223, 235, Pl. XX, A and B, and Pl. XXI, E), but differs mainly by the absence of oval, continuous conidia on aërial mycelium, and by the absence of chlamydospores.[61]

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[61] Chlamydospores were searched for very carefully in cultures on different media and of different ages, very old cultures being included, but were never observed. According to Wollenweber (1913 c: 208) such organisms, Ramularia-like but without chlamydospores, produce perfect forms of the genus Mycosphaerella; although the organism described here under the name of *R. Solani*, when grown on various media, did not produce chlamydospores, nevertheless it has been retained in this genus because of its otherwise perfect resemblance to the other unquestionable species of Ramularia.

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Submitted for publication February 10, 1915.

EXPLANATION OF PLATES

All the plates were reproduced by three-color process from living cultures. The cultures were in all cases, unless otherwise specified, forty days old when photographed, and were grown at laboratory room temperature ranging from 20° to 25° C.

Cultures of Plates I to IV were, with a few specified exceptions, grown on hard potato agar with 10 per cent glucose, and were kept in a strong diffuse daylight and in air nearly saturated with moisture.

All cultures of Plates V to VII were on sterilized potato tuber plugs in test tubes which were kept at the above temperature but in very weak diffuse light.

PLATE I. FUSARIA OF POTATOES

Fig. 1. Fusarium Solani, from above
Fig. 2. F. striatum, from above
Figs. 3 and 4. F. Martii var. minus: 3, on acidified medium from below; 4, on hard oat agar from above
Figs. 5 and 6. F. coeruleum, from above and below
Figs. 7 and 8. F. oxysporum var. resupinatum, from above and below
Figs. 9 and 10. F. lutulatum var. zonatum, from above and below
Figs. 11 and 12. F. sclerotioides, in dark, from above and below

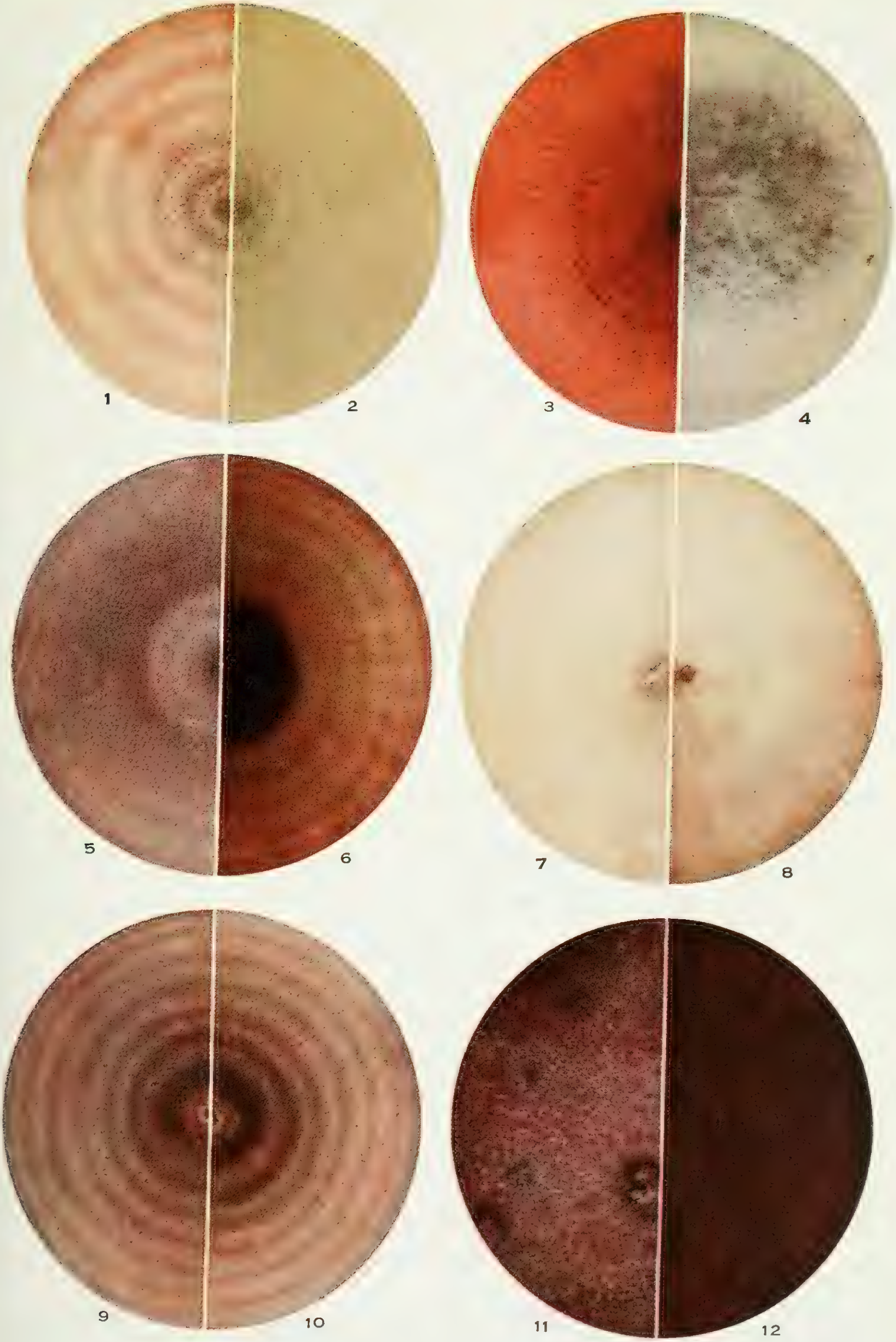

PLATE I—FUSARIA OF POTATOES

PLATE II. FUSARIA OF POTATOES

Figs. 1 and 2. Fusarium sclerotioides var. brevius, from above and below; 1, culture fifteen days old

Figs. 3 and 4. F. redolens var. Solani, from above and below

Figs. 5 and 6. F. lutulatum, from above and below; 6, culture on slightly acidified medium fifteen days old

Figs. 7 and 8. F. arcuosporum, from above and below

Figs. 9 and 10. F. lucidum, from above and below; fig. 9, culture fifteen days old, in dark

Fig. 11. F. subulatum, from above; hard oat agar

Fig. 12. F. subulatum var. brevius, from below, on slightly acidified medium

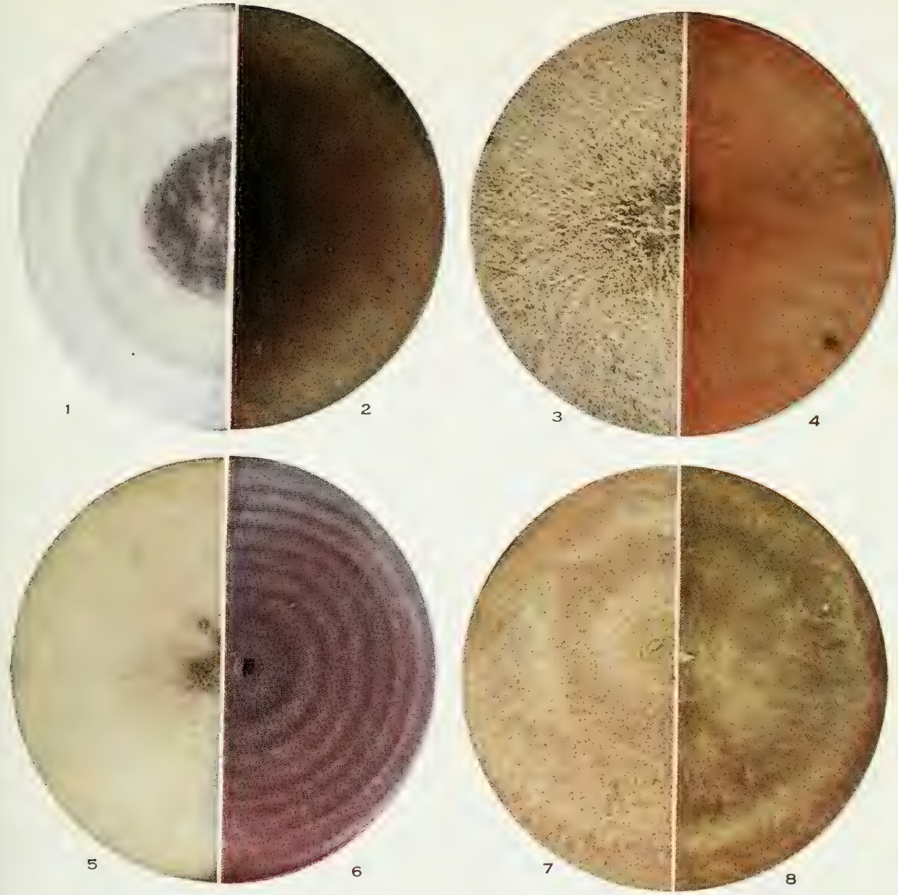

PLATE II—FUSARIA OF POTATOES

PLATE III. FUSARIA OF POTATOES

Fig. 1. Fusarium sporotrichioides, from above; in dark
Fig. 2. F. bullatum var. roseum, from above
Figs. 3 and 4. F. arthrosporioides, from above and below; in dark
Figs. 5 and 6. F. sanguineum var. pallidium, from above and below
Figs. 7 and 8. F. sanguineum, from above and below; in dark
Figs. 9 and 10. F. ferruginosum, from above and below
Figs. 11 and 12. F. clavatum, from above and below

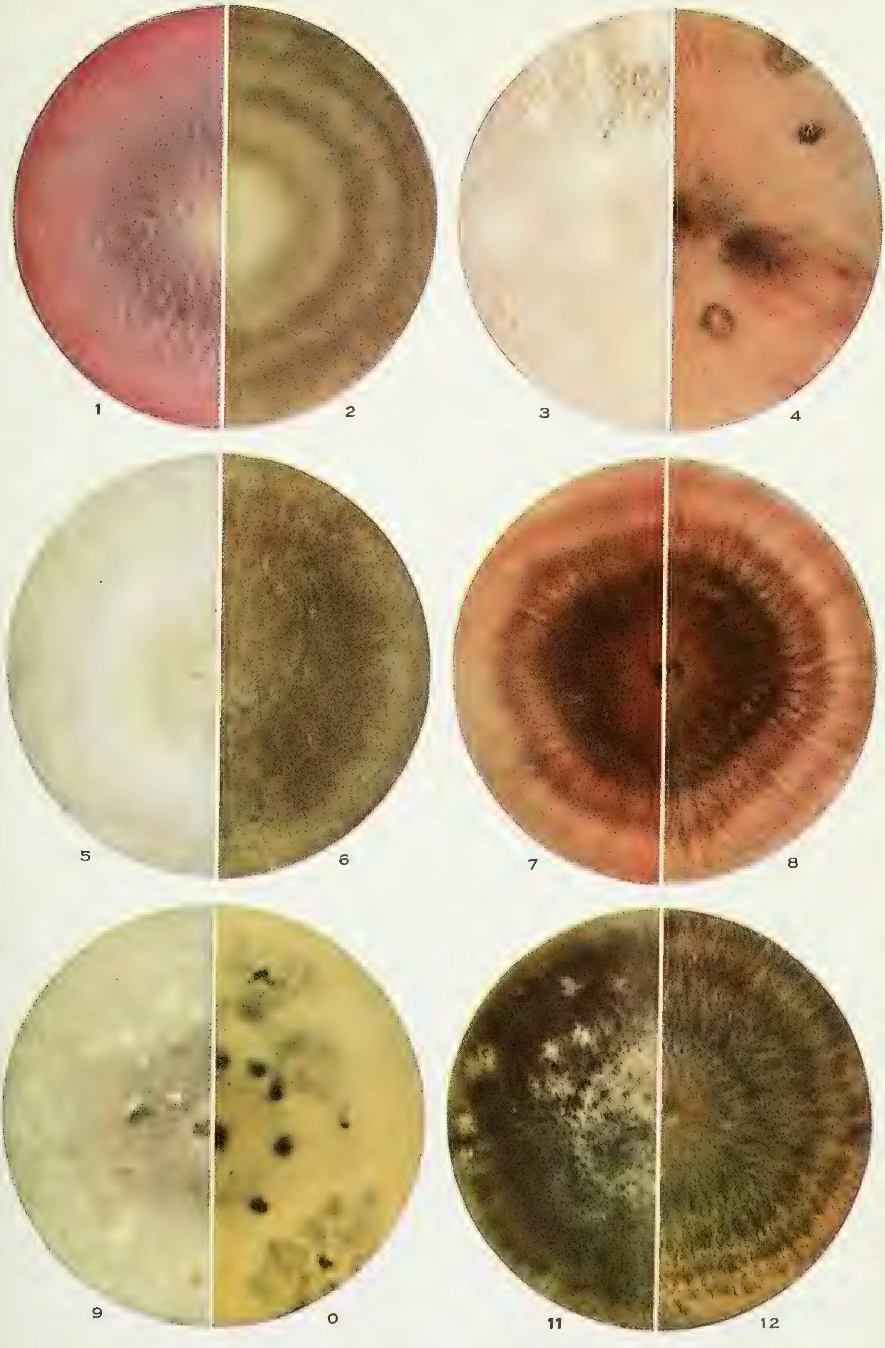

PLATE III—FUSARIA OF POTATOES

PLATE IV. FUSARIA OF POTATOES

Figs. 1 and 2. Fusarium culmorum var. leteius, from above and below
Fig. 3. F. subpallidum var. roseum, from above; on slightly acidified medium
Fig. 4. F. discolor, from below
Figs. 5 and 6. F. discolor var. triseptatum: 5, from above, in dark on neutral medium; 6, from above, in light on slightly acidified medium
Fig. 7. F. caudatum var. Solani, from above, neutral medium
Fig. 8. F. trichothecioides, from above
Fig. 9. F. culmorum from above, on hard oat agar
Fig. 10. F. culmorum var. leteius, from above, on hard oat agar
Fig. 11. F. discolor var. sulphureum, from above
Fig. 12. F. lucidum, from above, slightly acidified medium, fifteen days old

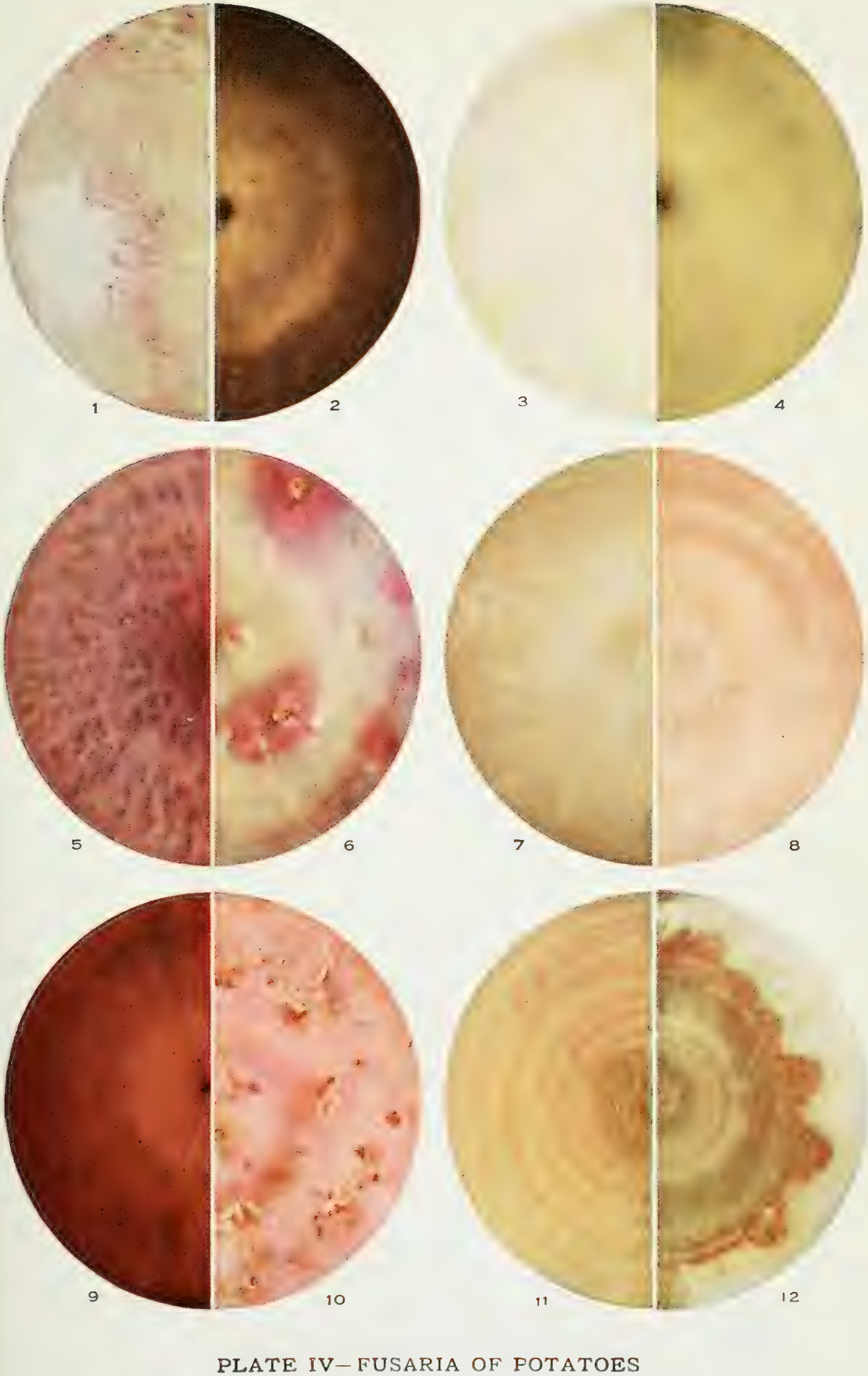

PLATE IV—FUSARIA OF POTATOES

PLATE V. FUSARIA OF POTATOES

Fig. 1. Fusarium sclerotioides
Fig. 2. F. redolens var. Solani
Fig. 3. F. lutulatum
Fig. 4. F. lutulatum var. zonatum
Fig. 5. F. oxysporum var. resupinatum
Fig. 6. F. oxysporum
Fig. 7. F. oxysporum var. asclerotium
Fig. 8. F. culmorum
Fig. 9. F. culmorum var. leteius
Fig. 10. F. discolor var. triseptatum
Fig. 11. F. discolor
Fig. 12. F. subpallidum

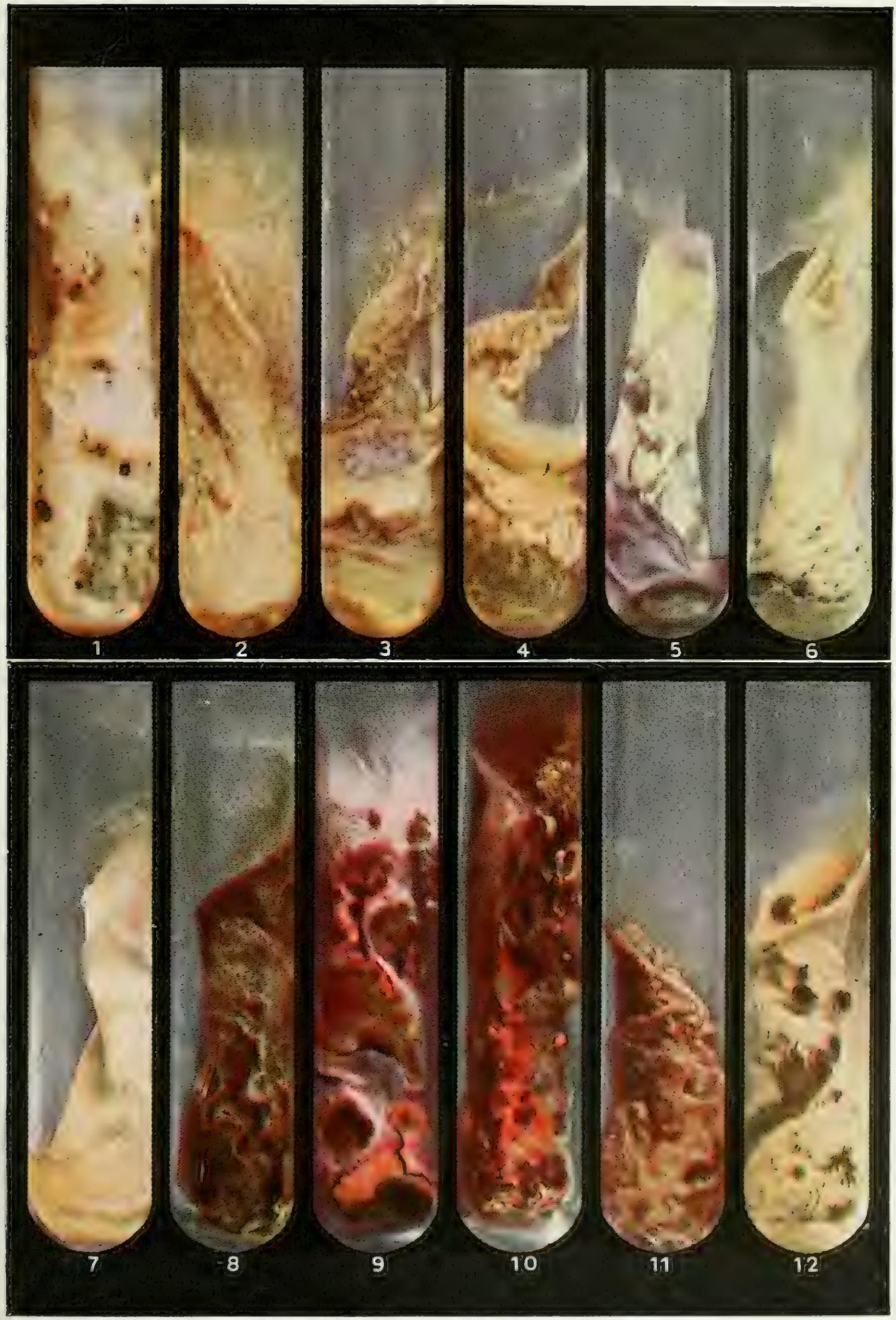

PLATE V—FUSARIA OF POTATOES

PLATE VI. FUSARIA OF POTATOES

Fig. 1. Fusarium sanguineum
Fig. 2. F. ferruginosum
Fig. 3. F. caudatum var. Solani
Fig. 4. F. coeruleum
Fig. 5. F. Martii var. viride
Fig. 6. F. Martii var. minus
Fig. 7. F. Solani
Fig. 8. F. radicicola
Fig. 9. F. anguioides var. caudatum
Fig. 10. F. arcuosporum
Fig. 11. F. anguioides
Fig. 12. F. lucidum

PLATE VI. FUSARIA OF POTATOES

Fig. 1. Fusarium sanguineum
Fig. 2. F. ferruginosum
Fig. 3. F. caudatum var. Solani
Fig. 4. F. coeruleum
Fig. 5. F. Martii var. viride
Fig. 6. F. Martii var. minus
Fig. 7. F. Solani
Fig. 8. F. radicicola
Fig. 9. F. anguioides var. caudatum
Fig. 10. F. arcuosporum
Fig. 11. F. anguioides.
Fig. 12. F. lucidum

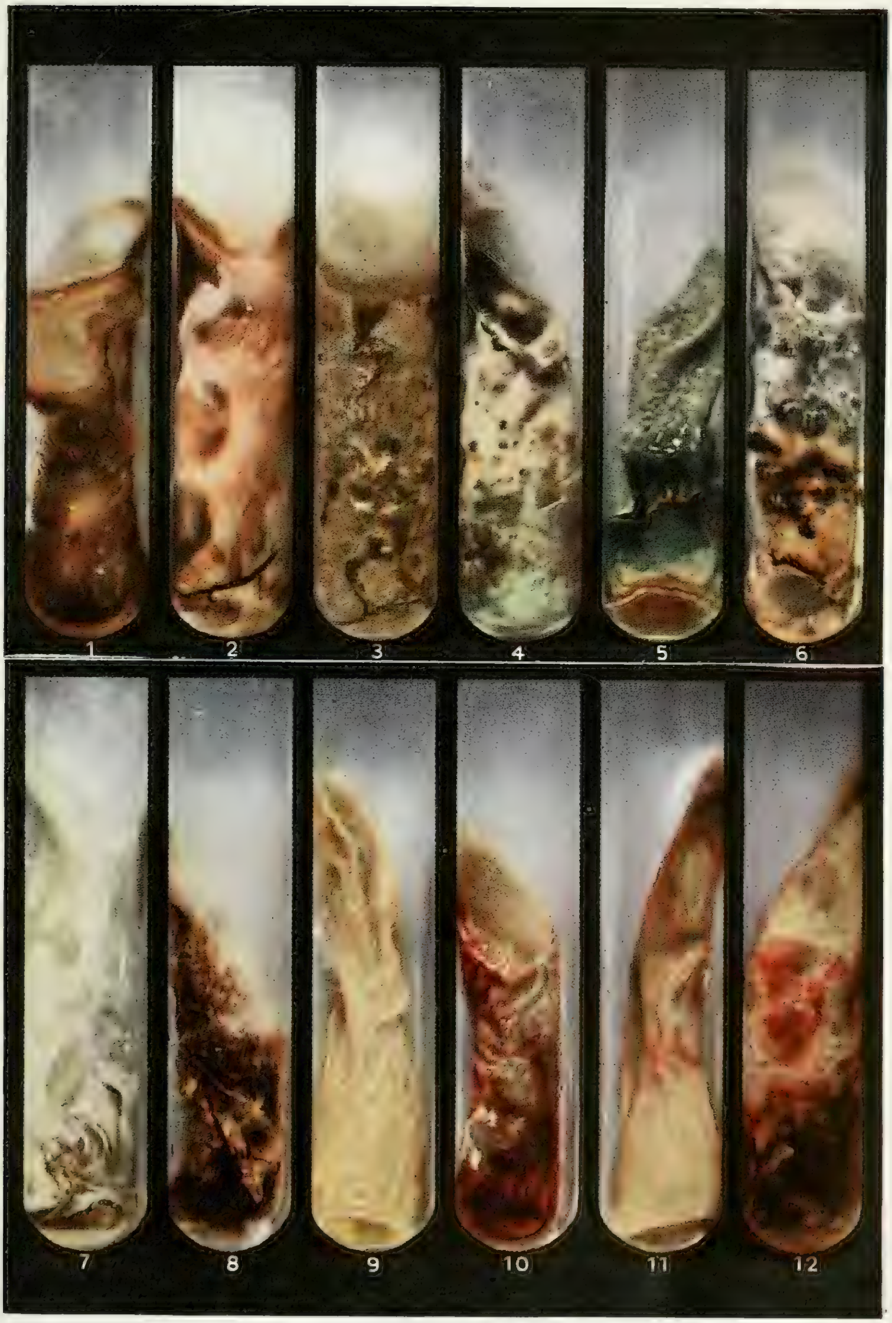

PLATE VI–FUSARIA OF POTATOES

PLATE VII. FUSARIA OF POTATOES

Fig. 1. Fusarium truncatum
Fig. 2. F. clavatum
Fig. 3. F. subulatum var. brevius
Fig. 4. F. subulatum
Fig. 5. F. metacroum
Fig. 6. F. effusum
Fig. 7. F. sanguineum var. pallidium
Fig. 8. F. falcatum var. fuscum
Fig. 9. F. arthrosporioides var. asporotrichius
Fig. 10. F. biforme
Fig. 11. F. arthrosporioides
Fig. 12. F. diversisporum, pseudopionnotal stage

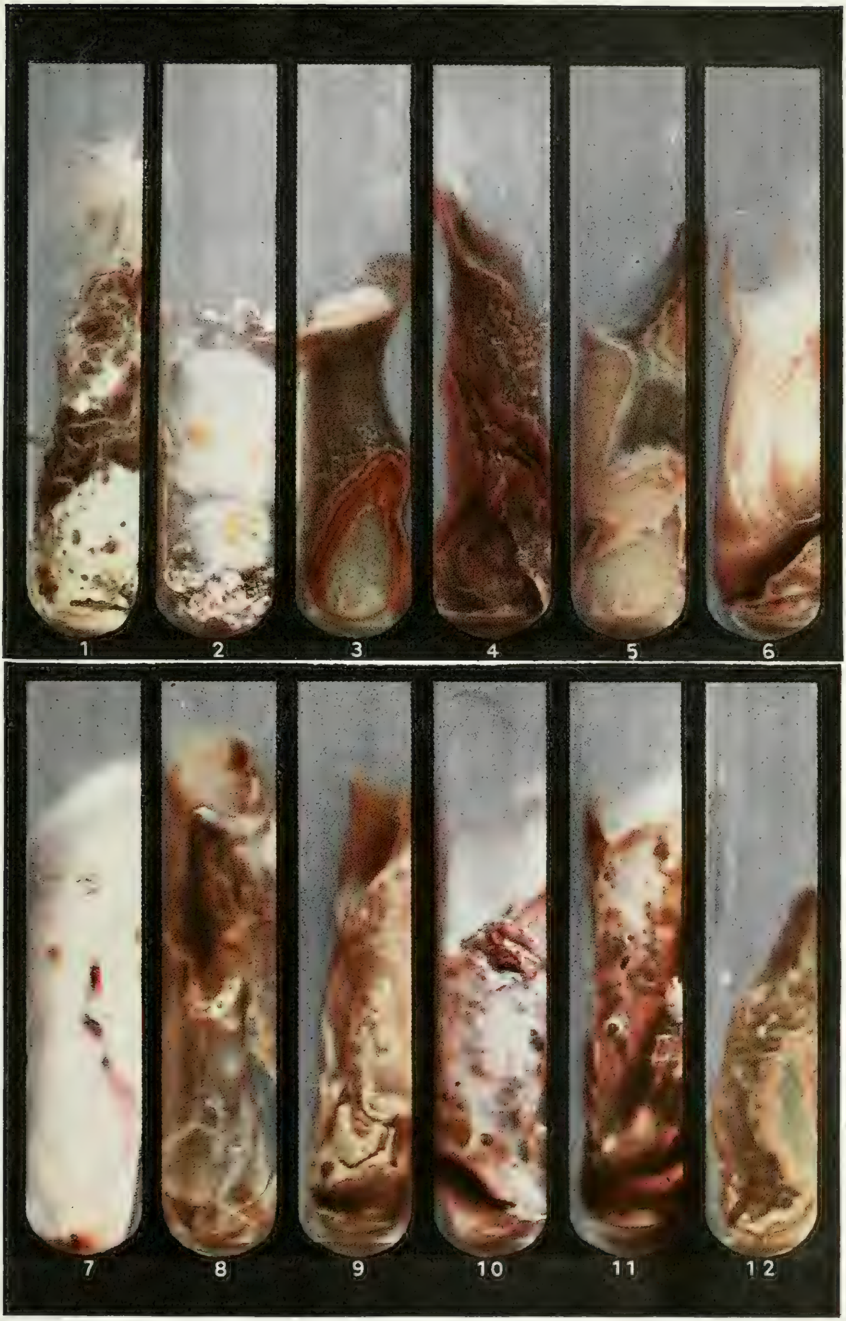

PLATE VII—FUSARIA OF POTATOES

JULY, 1915 MEMOIR No. 8

CORNELL UNIVERSITY
AGRICULTURAL EXPERIMENT STATION
OF THE COLLEGE OF AGRICULTURE

A BACTERIAL DISEASE OF STONE FRUITS

By F. M. ROLFS
FORMERLY OF THE DEPARTMENT OF PLANT PATHOLOGY, CORNELL UNIVERSITY
NOW PROFESSOR OF BACTERIOLOGY, CLEMSON COLLEGE, SOUTH CAROLINA

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A BACTERIAL DISEASE OF STONE FRUITS

A BACTERIAL DISEASE OF STONE FRUITS[1]

F. M. Rolfs

HOST CONSIDERATIONS

Cultivated varieties of the apricot (*Prunus armeniaca*), the nectarine (*Prunus persica* var. *necturina*), the peach (*Prunus persica*), and the plum (*Prunus domestica*, *Prunus americana*, and others), are known to be affected by the bacterial disease discussed herein. It will be noted that the hosts are closely related.

THE DISEASE

NAMES

The disease on the foliage during its early development or in cases cf slight infection is known as *bacterial leaf spot;* in more advanced stages or in more serious outbreaks it is termed *bacterial shot hole.* On the fruit it is sometimes referred to as *black spot*, the growers, however, more commonly speaking of it as *bacterial crack.* The disease on the young twigs has received the names *black spot* and *black tip*, and when open perennial wounds are formed on the branches these are called *bacterial cankers.*

HISTORICAL

The disease is apparently of American origin, it having been found, so far as is known, only in the United States. During the past nine or ten years fruit growers in various sections of the country have called attention to a shot-hole disease of peach and plum foliage, which in wet seasons has in many cases caused premature defoliation. O'Gara (Rorer, 1909)[2] reports a shot-hole condition of peach foliage prevalent in Georgia, and suggests that it is caused by a bacterial organism. Smith (1903) calls attention to a bacterial disease of Japanese varieties of plum in central Michigan, which appears as a shot hole of the leaves and a black spot of the fruit. Clinton (1904) describes a leaf spot on peach in Connecticut, which he attributes to a bacterial organism. Heald (1906) discusses a disease, apparently of the same nature, found in Nebraska on the twigs

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[2] Dates refer to literature cited, page 435.

and branches of Wickson and Whitaker varieties of plum. Jackson (1907) reports a similar disease from Delaware, and states that considerable injury results to the plum orchards in that State. Smith (1905 b, figs. 11, 12, 15, 70, 72, pl. 19) gives diagrams of sections through diseased plum leaves and fruit tissue, and illustrations of diseased plum fruit. Rorer (1909) records a detailed study of the disease on leaves, twigs, and fruits of peach. Smith (1911) discusses the disease on plum leaves and fruits, and gives illustrations of diseased plum fruits as well as sections through diseased fruit tissue. Heald and Wolf (1912) report the occurrence of a bacterial canker on plum at San Antonio, Texas. Lewis (1912) describes a bacterial canker on plum twigs and records the results of several inoculation experiments. He gives illustrations of cankers on twigs four and six years of age.

The disease was first brought to the attention of the writer in 1904, when he was Botanist and Horticulturist at the University of Florida. Many letters were received from fruit growers in northern Florida and southern Georgia, complaining of a shot-hole condition of peach and plum leaves accompanied by considerable premature defoliation. The foliage of the different varieties of peach and plum trees in the university horticultural orchard also developed the shot-hole condition, and in most cases fell prematurely. Other duties prevented the writer from undertaking extensive investigation of the problem at that time. However, on taking up work at the Missouri State Fruit Experiment Station in 1906, he found that the blackened areas, so common on the peach twigs in northern Florida in 1905, were even more abundant on the peach and plum twigs in Missouri. Additional observations have brought out the fact that the disease is more or less injurious also to the apricot and the nectarine.

GEOGRAPHICAL DISTRIBUTION

It is not easy to obtain information as to the distribution of this disease, since a bibliography of the literature relating to shot-hole injury of stone fruits shows that the injury is usually attributed to the work of fungi. This is not surprising, as lesions resulting from various causes resemble one another so closely that it is often impossible to determine exactly the causal factor without the aid of a microscope, and it is not always possible even with a microscope. The disease has been reported by investigators as occurring in Arkansas, Connecticut, Delaware, Georgia, Michigan, Nebraska, and Texas. Diseased material has been obtained by the writer from Alabama, Arkansas, Florida, Georgia, Illinois, Iowa, Kansas, Michigan, Missouri, New Jersey, New York, Ohio, Oklahoma, Pennsylvania, South Carolina, Tennessee, and Texas.

ECONOMIC IMPORTANCE

Bacterial leaf spot, or shot-hole as it is usually called, is one of the chief foliage diseases of stone fruits in the humid districts of the South. It occasionally becomes more or less aggressive also in some sections of the northern and eastern States. Its importance varies from year to year, there being an abundance in wet seasons and little in dry seasons. The disease appears to be more severe in warm, humid regions than in cool, dry sections. In southern Missouri its economic importance has kept pace with the growth of the peach industry, and it is now one of the most difficult diseases with which the fruit grower has to contend. Its attacks on the plum have made it practically impossible to grow certain varieties of Japanese plums on a commercial scale. The fruit of some varieties of apricot and nectarine also are injured more or less by the disease, but since apricots and nectarines are not extensively grown in Missouri the disease on these fruits has not attracted much attention.

In different parts of Missouri, during 1908 and 1909, a record was made of the amount of infected fruit in diseased Elberta peach orchards, in order to determine the extent of the injury due to this disease. It was found that from 1 to 10 per cent of the fruit was infected in orchards well cared for, and from 25 to 75 per cent in orchards poorly cared for. The lesions on the fruit are in many cases not sufficiently prominent to mar its appearance greatly, but the larger proportion of such fruit is graded as "seconds." None of the fruit holds up well in shipping, and in some cases it is completely destroyed by various rot-producing organisms before being placed on the market.

It is especially difficult to obtain reliable figures on the loss due to this disease, since the loss is not confined to the direct effect on the fruit but must include also the influence of the diseased foliage on the general vigor of the tree. Severe outbreaks soon impair the efficiency of the foliage, and if they occur early in the season they reduce the size and greatly lower the quality of the fruit. Severe foliage injury also has a marked influence on the formation of fruit buds, and trees that have their leaves injured early in the season usually carry a very light crop of fruit the following year. Young trees are usually more affected than those six or seven years old, and badly diseased young trees soon become stunted and in some cases permanently injured.

SYMPTOMS

The disease affects the leaves, twigs, and fruit in a characteristic manner. It develops on twigs and shoots of only the current year's growth, but the wounds on the twigs often persist as open perennial cankers.

The very common fungous disease of apricot and plum foliage due to *Cylindrosporium padi* is similar in its symptoms to bacterial leaf spot, but usually can be distinguished from the latter by the white downy growth on the lower surface of the spot. Pierce (1894) reports a similar disease on almond foliage in California due to the work of *Cercospora circumscissa*. There is also a leaf spot of unknown cause which is constantly associated with peach and plum rosette. Spray mixtures and other toxic substances applied to the leaves often produce an injury which is difficult, without the aid of a microscope, to distinguish from bacterial leaf spot. Care must be taken not to confuse the lesions due to these various agents.

On the leaves

The first evidence of the disease on the leaves is the appearance of small gray specks (Fig. 59), which become somewhat angular in form and soon take on a water-soaked appearance. They later become brown or purplish brown, in some cases scarlet, and finally change to dark brown. The spots measure from one to five millimeters in diameter and when numerous they coalesce, so that large areas of the leaf may be involved. A more or less circular diseased area is finally separated by a line of cleavage from the healthy tissue, the injured tissue within this area promptly contracting, drying, and falling out. This gives the leaf the shot-hole appearance. On the plum, in some cases, after the disease has apparently run its course and the spot is well formed, a second and even a third period of invasion may follow and an area one-half inch or more in diameter may develop from a single spot. Leaves that are badly diseased soon fall to the ground. It is not unusual for an infected tree to lose from fifteen to twenty-five per cent of its leaves by August, and in the more extreme cases as high as seventy-five per cent, or even more, of the leaves

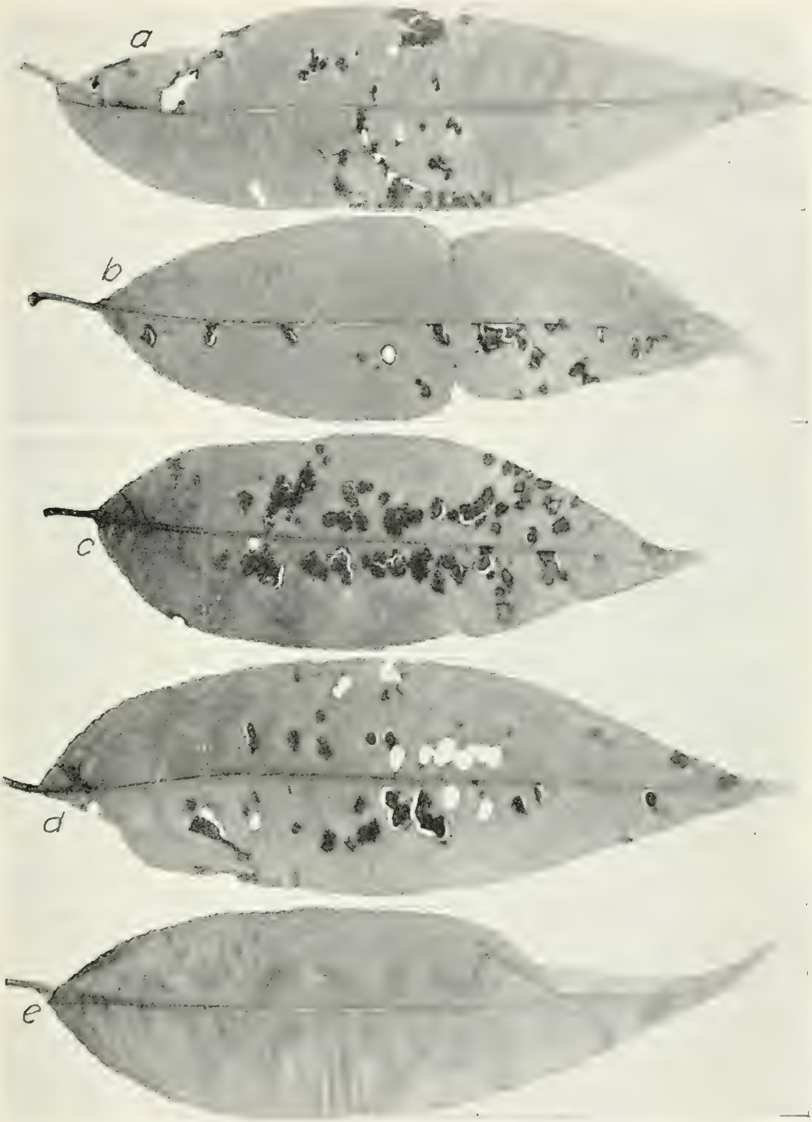

Fig. 59.—*Elberta peach leaves inoculated with pure cultures of Bacterium Pruni. The growth from a seven-days-old-slant Hiss-glucose culture was thoroughly mixed in one hundred cubic centimeters of sterile water and applied to the leaves by dipping the fingers into the water and then rubbing the leaf between the thumb and the fingers. Inoculations were made on July 16, 1912, and the photograph was taken on August 28, 1912*

a, Inoculated with a pure culture obtained from an apricot twig
b, Inoculated with a pure culture obtained from a nectarine twig
c, Inoculated with a pure culture obtained from a peach twig
d, Inoculated with a pure culture obtained from a plum twig
e, Check. Sterile water was applied to the leaf

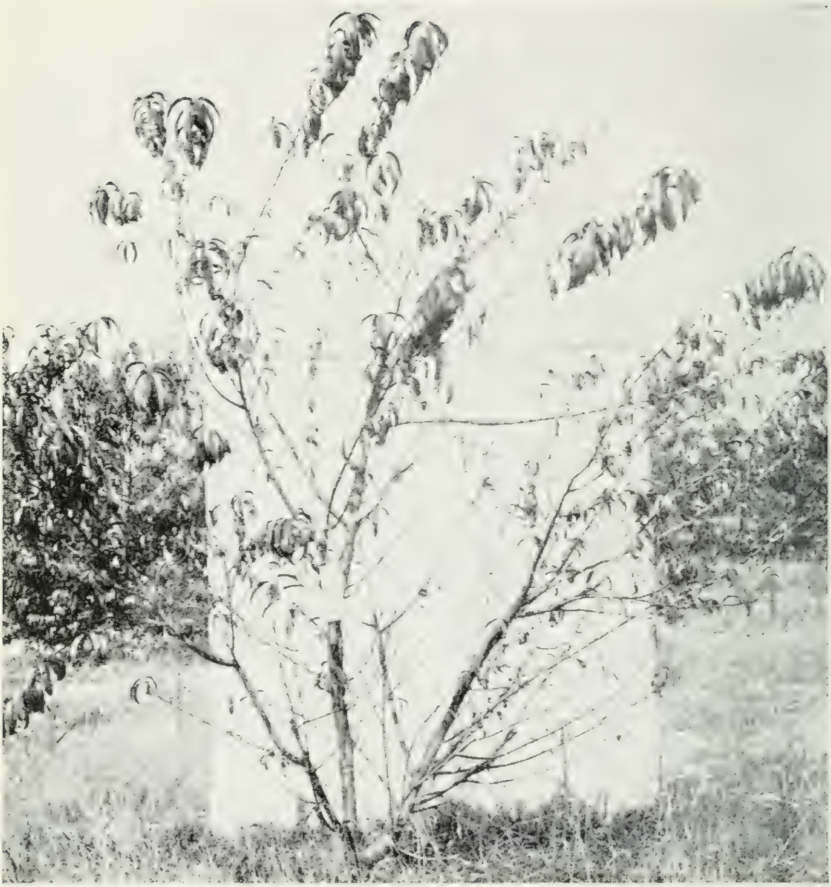

Fig. 60.—*Arp Beauty peach tree showing defoliation due to the work of Bacterium Pruni. Most of the leaves that formed before July 1, 1909, have fallen. All leaves still on the tree are more or less injured.* (*Photograph made on September 6, 1909*)

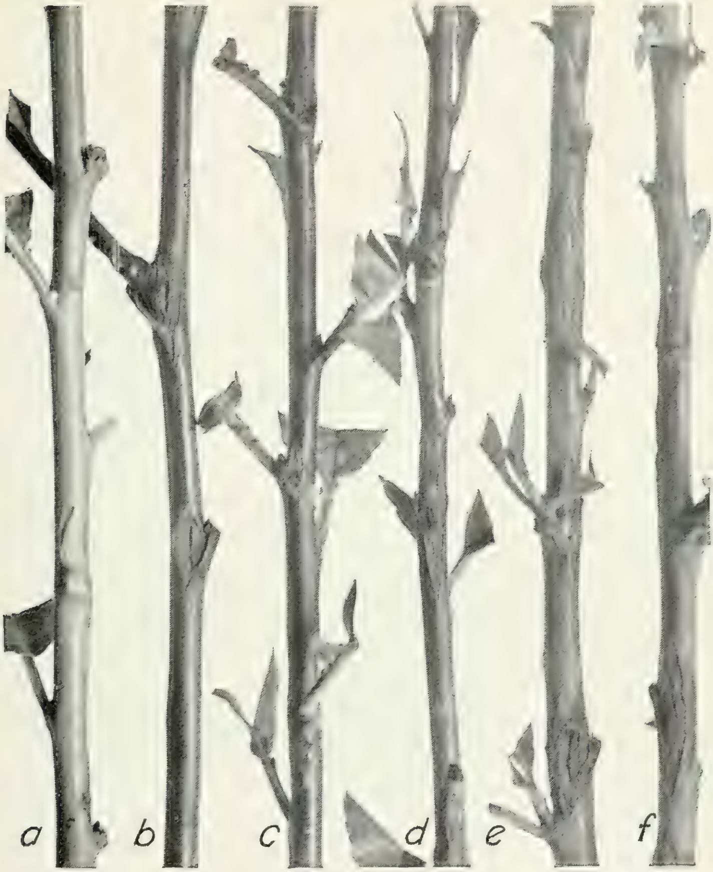

FIG. 61.—*Elberta peach twigs inoculated with pure cultures of Bacterium Pruni. The growth from an eight-days-old-slant Hiss-glucose culture was thoroughly mixed in one hundred cubic centimeters of sterile water. Each twig was inoculated at four points by inserting the point of a hypodermic needle just below the cuticle and forcing the water containing the organism into the tissues. Inoculations were made on June 25, 1912, and the photograph was taken on August 20, 1912*

a. Check. Sterile water was used
b and c, Inoculated with a pure culture obtained from an apricot twig
d, Inoculated with a pure culture obtained from a nectarine twig
e, Inoculated with a pure culture obtained from a peach twig
f, Inoculated with a pure culture obtained from a plum twig

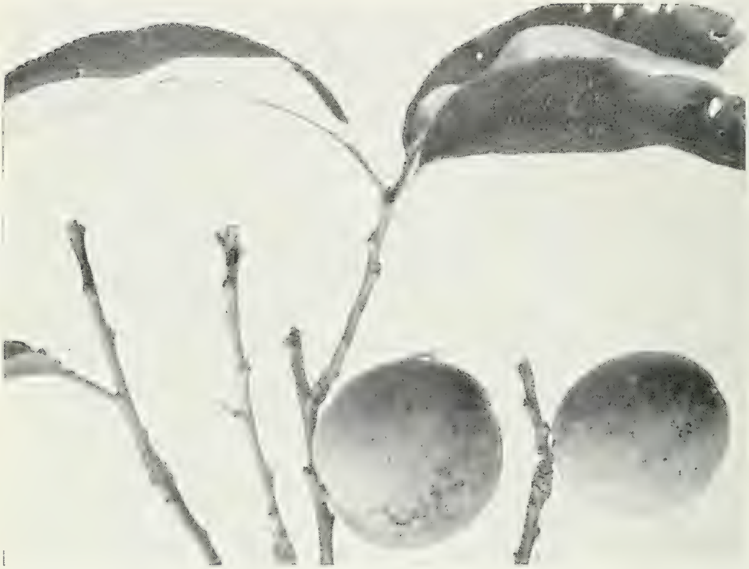

Fig. 62.—*Elberta peach twigs, leaves, and fruit, showing injury by Bacterium Pruni. The black tips of the twigs are to be noted particularly.* (*Photograph made on August 15, 1909*)

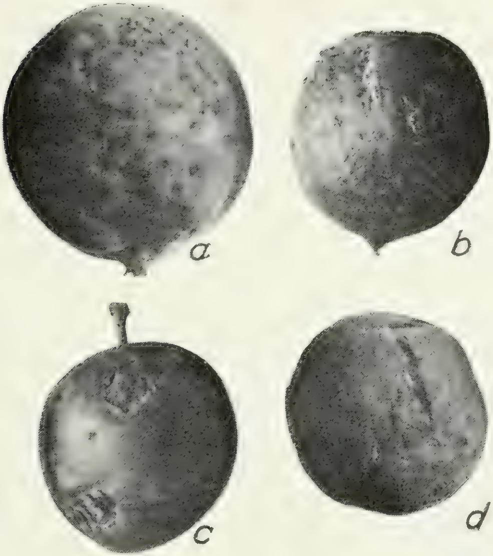

Fig. 63.—*Naturally infected fruits of peach, nectarine, plum, and apricot: a, Crothier peach; b, Victoria nectarine; c, Abundance plum; d, Bread apricot. (Photograph made on July 1, 1909)*

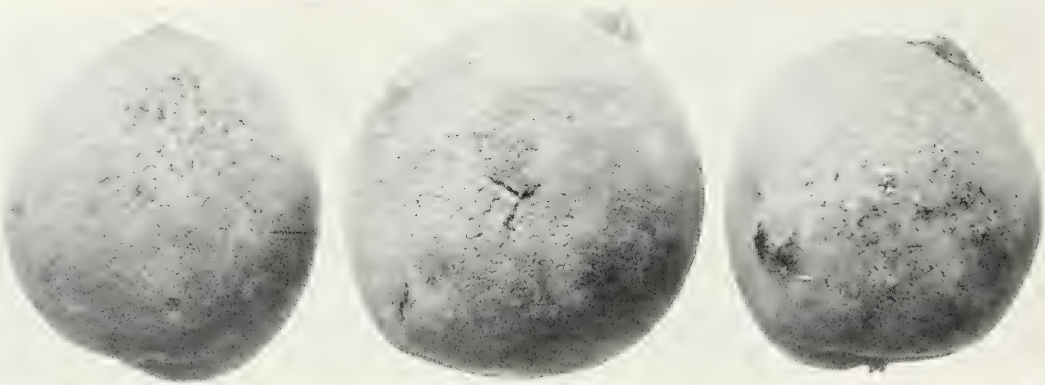

Fig. 64.—*Elberta peach fruit showing injury by Bacterium Pruni. The tree was sprayed seven times. The first application was made with lime-sulfur solution, 15-15-50 formula, on March 12, shortly before the fruit buds had opened. The other six applications were made with self-boiled lime-sulfur mixture, 12-8-50 formula. The first of these applications was made on April 21, just after the leaf buds opened; the others followed at intervals of about twelve days. Fifty-two per cent of the fruit was dotted with numerous cracks. (Photograph made on August 12, 1908)*

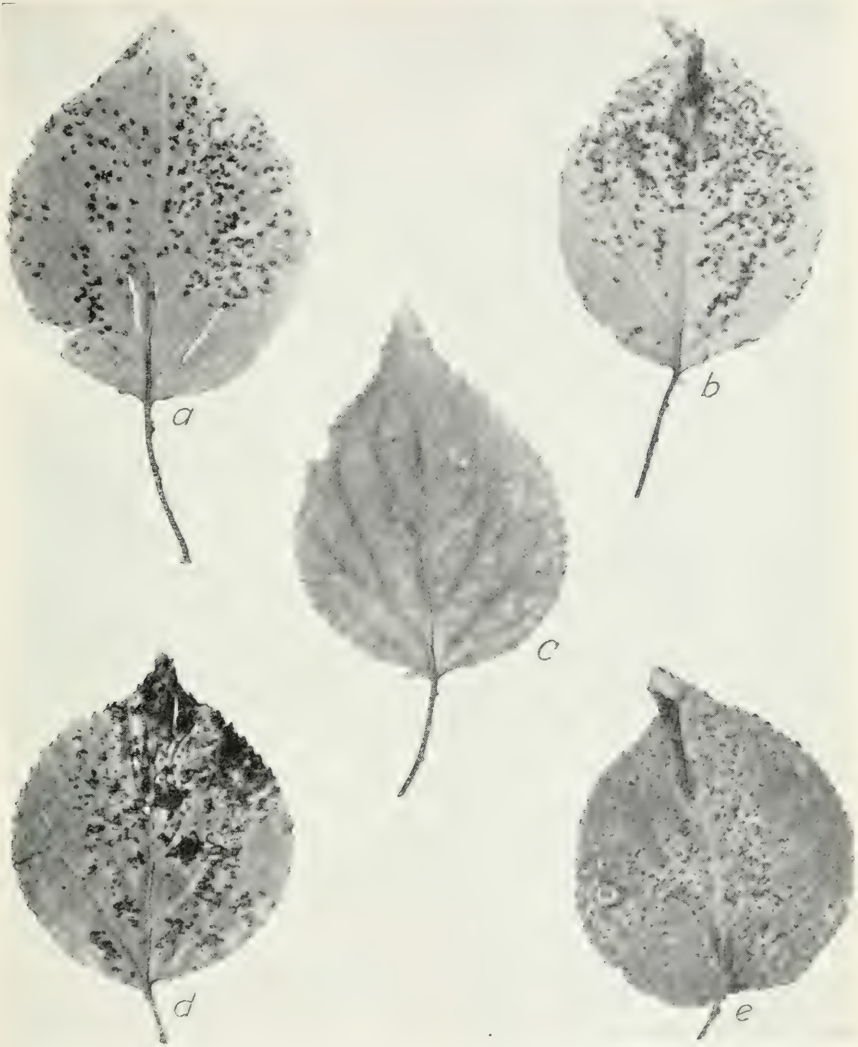

FIG. 65.—*Alexander apricot leaves inoculated with pure cultures of Bacterium Pruni. The growth from a six-days-old-slant Hiss-glucose culture was thoroughly mixed in one hundred cubic centimeters of sterile water and then applied to the leaves by dipping the fingers into the water and gently rubbing the leaves between thumb and fingers. Inoculations were made on June 29, 1912, and the photograph was made on August 27, 1912*

a, Inoculated with a pure culture obtained from an apricot twig
b, Inoculated with a pure culture obtained from a nectarine twig
c, Check. Sterile water was applied to the leaves
d, Inoculated with a pure culture obtained from a peach twig
e, Inoculated with a pure culture obtained from a plum twig

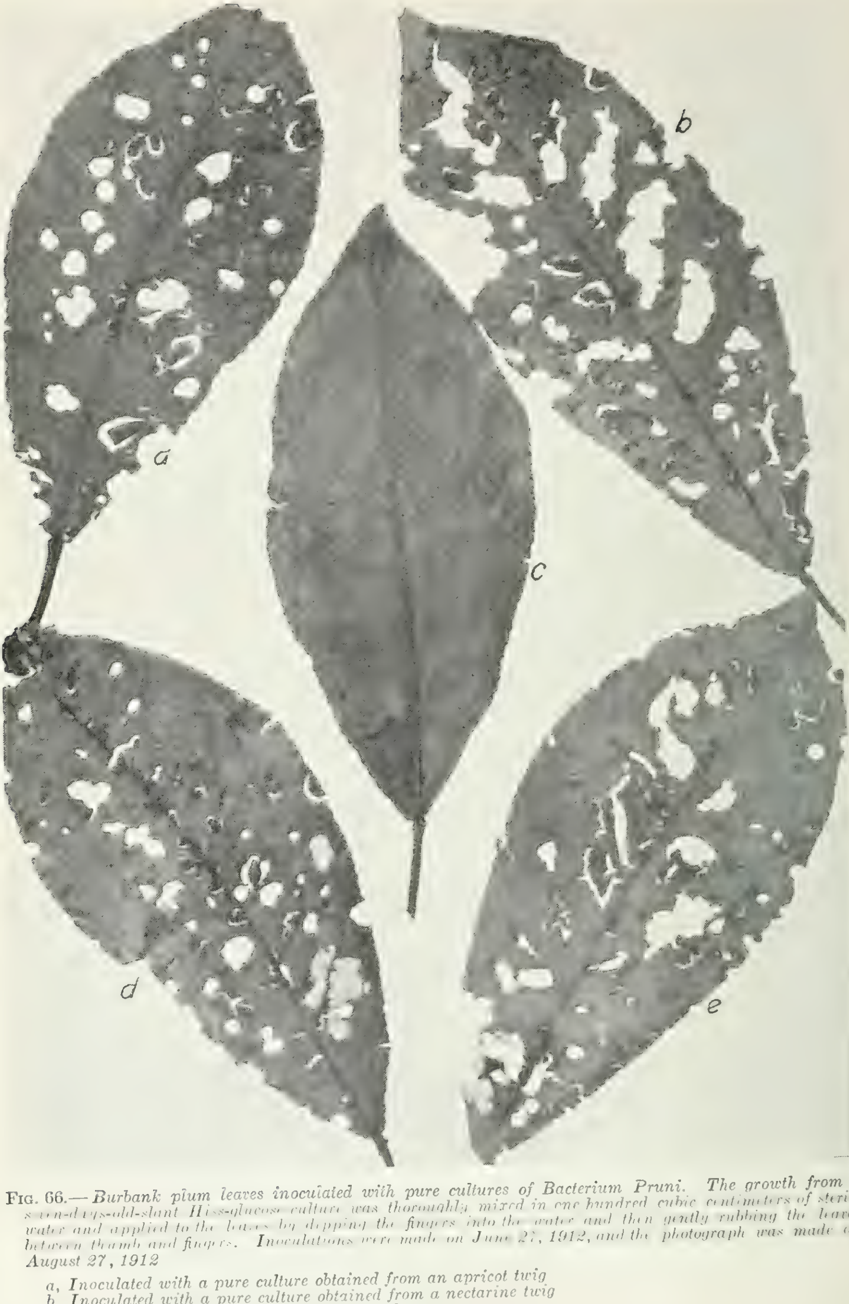

Fig. 66.— *Burbank plum leaves inoculated with pure cultures of Bacterium Pruni. The growth from a seven-days-old-slant Hiss-glucose culture was thoroughly mixed in one hundred cubic centimeters of sterile water and applied to the leaves by dipping the fingers into the water and then gently rubbing the leaves between thumb and fingers. Inoculations were made on June 27, 1912, and the photograph was made on August 27, 1912*

a, Inoculated with a pure culture obtained from an apricot twig
b, Inoculated with a pure culture obtained from a nectarine twig
c, Check. Sterile water was applied to the leaves
d, Inoculated with a pure culture obtained from a peach twig
e. Inoculated with a pure culture obtained from a plum twig

fall. The older, first infected leaves are usually, although not always, the first to go. One leaf after another falls, leaving finally only a tuft of young leaves at the tip of each twig and giving the tree a characteristic appearance (Fig. 60).

On the twigs

In some seasons black cankers form on the twigs in early spring, but as a rule these do not appear in abundance until May or June. The first visible indication of the canker is the appearance on the young shoots of a water-soaked spot surrounding a lenticel. In some cases the diseased area bulges out, more or less, in the beginning. As the spot enlarges it soon elongates. Single spots measure from one to four centimeters in length and often extend from one-half to two-thirds of the way around the shoot. The area becomes more or less sunken in age, soon becomes brown or purplish brown, then dark brown, and finally purplish black or black (Fig. 61). It frequently happens, especially on the nectarine and the peach, that several of the spots become confluent, forming areas which extend from two to three inches along the stem and in some cases completely girdle it. In favorable seasons the tips of many of the shoots are entirely blackened and killed (Fig. 62), this condition being most prominent on the nectarine and the peach. In most cases the injury is overcome gradually, and as a rule all traces of these black cankerous spots disappear in the second year.

The black spots on apricot twigs also disappear for the most part, but on plum twigs many of them persist from year to year, forming perennial cankers. As many as twenty-two cankers in different stages of development have been counted on a single plum twig (a water sprout). Open cankers also form on the apricot, the nectarine, and the peach, but they are far less abundant on these trees than on the plum.

The disease is usually more aggressive during the first two or three years after planting than it is later. It is not only likely to defoliate the young trees prematurely, but in many instances it kills back the twigs and frequently stunts the trees. A case of this sort was under observation, in which two hundred Elberta peach trees from an infected nursery were mixed with a shipment of twenty-three thousand healthy trees. Within three years from the time of planting, every tree in the orchard was more or less diseased, and many of the trees remained small and produced small, inferior fruit.

On the fruit

The disease does not ordinarily appear on the fruit until about the middle of May. Injured fruits of all the four kinds of hosts, however, have been collected early in May (Fig. 63). The first external signs of the disease are numerous small gray specks just below the cuticle. These specks enlarge slightly and soon take on a water-soaked appearance or a purplish color. The skin over the spots is soon ruptured, producing numerous straight or angular cracks which resemble the wounds on chapped hands (Fig. 64). Usually individual spots do not measure much more than two millimeters in diameter, but they are often so numerous that the cracks soon become confluent and frequently form a network, fissures nearly an inch in length being developed in some cases. On young growing fruit it is not uncommon to find rifts extending nearly the entire length of the fruit. Such wounds mar its appearance and in many cases render it worthless for market.

On all kinds of fruit the disease results in the formation of these small, more or less abundant, crack-like wounds, but certain varieties of the nectarine are especially liable to this type of injury. In some cases the spots gradually widen and extend for a considerable depth into the tissue, forming roundish, sunken, black spots which finally measure from one-fourth to one-half inch in diameter. Rifts develop in the blackened tissue. These widen, and cracks appear throughout the diseased area which often (especially when the disease is on young developing fruit) finally extend beyond the dead into the healthy tissue. In many cases the fruit becomes so badly distorted and cracked that it is worthless, and it is soon destroyed by various rot-producing organisms.

ETIOLOGY

Historical

The pathogen causing this disease was first isolated by the writer in 1904 at the University of Florida. Many poured plate cultures were made from the spots on injured leaves of both peach and plum. Cultures made from the young water-soaked spots invariably gave numerous yellow bacterial colonies, and in no case was a species of Cercospora or Cylindrosporium obtained. Consequently it was concluded that the disease was of bacterial origin. It was observed also that the defoliated twigs sometimes showed one or more blackened, slightly sunken areas. These often measured as much as seven millimeters in width and extended from five to fifty millimeters along the stem, in some cases the entire end of the twig being blackened. Cultures from these areas produced yellow bacterial colonies.

On taking up work at the Missouri State Fruit Experiment Station in 1906, the writer observed that the twigs of some varieties of peach and plum in Missouri also showed blackened, sunken areas, and poured plate cultures from the diseased tissue in many cases gave yellow bacterial colonies. About the middle of June, 1906, it was observed that the foliage of trees bearing the diseased twigs had developed the shot-hole condition to a marked extent, while the foliage on trees with twigs free from the blackened areas was quite free from shot-hole. The fruit on Abundance and Burbank plum soon developed many typical black-spot areas, from which pure cultures of a yellow organism were easily obtained. A little later many peach fruits developed numerous small cracks. These were at first supposed to be due to the work of curculio, but a careful examination showed bacteria to be present in enormous numbers.

A careful study soon proved that the organism had invaded the apricot and the nectarine in much the same way as it had the peach and the plum. The fruit of the nectarine in the station variety orchard in 1906 was so badly cracked as a result of the attack of this organism that the entire crop was destroyed by various decay organisms long before the ripening period. Some of the apricot fruits developed the typical black spot. The twig cankers on both apricot and nectarine were fully as plentiful as on the peach, and premature defoliation also occurred on both apricot and nectarine trees.

Nomenclature

Since bacteriologists have not yet adopted an international code of nomenclature, it does not seem advisable to enter here into an extended discussion of the limits of genera, the selection of types, or the position of this organism in the various systems of classification proposed. The writer has therefore elected to follow arbitrarily the suggestion given by Erwin F. Smith (1905a). The organism was first described by Smith (1903) under the name *Pseudomonas Pruni*. Later, however, it became *Bacterium Pruni*, when Smith substituted the generic name Bacterium Cohn emend. for Pseudomonas Migula.

Pathogenicity

Conclusive proof of the pathogenic nature of this organism was obtained in 1904 by Smith (1905 a), by inoculating the foliage and the fruit of Abundance plum. The pure culture of the organism used in inoculation was obtained from plum fruit of the same variety. The organism from several young-slant agar cultures was scraped into two hundred cubic centimeters of sterile water and sprayed with an atomizer on the foliage and fruit of a five-years-old tree. Diseased spots developed on both leaves and fruit in from ten to twelve days and became numerous after fourteen days. Poured plates from the fruit and the leaves of the inoculated trees yielded numerous colonies of the yellow organism. In 1907 Smith (1908) also successfully inoculated the foliage of a peach tree with the plum organism. Rorer (1909) isolated the organism from peach leaves in 1906, and the following year made a series of successful inoculations with it on the foliage of Elberta peach trees at Bentonville, Arkansas. His results agree in every way with those obtained by Smith with the plum organism. Rorer also obtained pure cultures of the organism from peach twigs and reported its occurrence on peach fruit. He found the cultural characters of the plum and peach organisms to be exactly the same.

In 1907, at Mountain Grove, Missouri, the writer repeated and confirmed Smith's experiments on the plum, and extended the infection experiments to apricot, nectarine, and peach. The organism used was isolated from an Abundance plum fruit and grown in pure culture. The organism was scraped from several slant agar cultures into one hundred cubic centimeters of sterile water, and sprayed with an atomizer on the fruit and leaves of eight-years-old trees of Royal apricot, Victoria nectarine, Elberta peach, and Abundance plum. The inoculations were made during a light, drizzling rain. Disease spots developed on fruit and leaves of all four hosts within twelve days, and after fifteen days they became very numerous. Poured plates from the fruit and the leaves of the inoculated trees yielded numerous colonies of the yellow organism. However, several of the check leaves on the apricot and the nectarine trees also developed spots from which the yellow organism was obtained. Pure cultures of the organism were obtained by the writer from leaves and twigs of apricot, nectarine, peach, and plum. These strains and the one obtained from an Abundance plum fruit were grown side by side on various culture media, and were exactly alike in all cultural characters.

In 1909, at Mountain Grove, cross inoculations with the four strains obtained from the leaves were made on eight-years-old Royal apricot, Victoria nectarine, Elberta peach, and Abundance plum trees. Two hundred leaves, fifty fruits, and twenty twigs were used in each case. The organism from young-slant Hiss-glucose cultures was thoroughly mixed with sterile water and sprayed on the leaves and the fruit with an atomizer. Each of the twenty twigs was inoculated at four points with a hypodermic syringe. Spots appeared on the leaves of all the inoculated trees in ten days, and on the fruit in from twelve to fifteen days. The inoculated tissue on the twigs assumed a water-soaked appearance in from four to eight days, becoming dark brown and finally, in about twenty-five days, almost black. Poured plate cultures from the spots on leaves, fruit, and twigs yielded numerous colonies of the yellow organism. Three of the check leaves on peach also developed a number of brown spots.

In 1910, at Mountain Grove, cross inoculations with strains of the organism from the fruit of the four hosts were made on eight-years-old Royal apricot, Victoria nectarine, Elberta peach, and Burbank plum trees. Fifty leaves and ten twigs were used in each case. The organism from young-slant Hiss-glucose cultures was scraped into sterile water. The fingers were dipped into this water, and the material was applied to the lower surface of the leaf by gently rubbing the leaf between thumb and fingers. Each twig was inoculated at six points with a hypodermic syringe. Gray spots appeared on the leaves of all trees in from six to seven days, and water-soaked spots appeared in great numbers about the tenth day. The inoculated tissue on the twig assumed a water-soaked appearance in from five to seven days and became dark brown in about twenty-five days. Poured plates from the spots on leaves and twigs yielded colonies of the organism more or less abundantly. All the twig check wounds soon healed, and the check leaves remained free from spots.

In 1911, at Ithaca, New York, cross inoculations with the four strains obtained from the twigs of the four different hosts were made on three-years-old Elberta peach and four-years-old Abundance plum trees. The organism from young-slant Hiss-glucose cultures was thoroughly mixed with sterile water and sprayed on the leaves with an atomizer. Twelve twigs were used in each case, and each twig was inoculated at five points with a hypodermic syringe. Spots on the leaves developed in nine days, and after twelve days water-soaked spots appeared abundantly. The inoculated tissue on the twigs assumed a water-soaked appearance in most cases by the seventh day. The typical black-spot and black-tip condition of the twig did not develop until after the twenty-fifth day. Poured plate cultures from the young leaf spots and the black spots on the twigs yielded colonies of the organism abundantly. Pure cultures of the organism from the twigs of the four kinds of trees were grown side by side on various culture media for six weeks, and in all cultural characters the strains were the same. All check wounds on the twigs soon healed, and the check leaves remained free from spots.

In 1912, at Ithaca, cross inoculations with strains of the organism from twigs of the four hosts were made on three-years-old Alexander apricot, Early Violet nectarine, Elberta peach, and Burbank plum trees (Figs. 59, 65, and 66). One hundred leaves and from twelve to twenty twigs were used in each case. The organism from young-slant Hiss-glucose cultures was scraped into sterile water. The fingers were then dipped into the water, and the material was applied to the lower surface of the leaf by gently rubbing the leaf between thumb and fingers. Each twig was inoculated at from three to five points with a hypodermic syringe (Fig. 61). Spots on the leaves appeared in from five to seven days, and water-soaked spots became numerous after the eighth day. The inoculated tissue on the twigs took on a water-soaked appearance in about seven days, but did not become dark brown until after the twenty-fifth day. Both leaf and twig spots yielded the characteristic yellow colonies on poured plate cultures. All check wounds on the twigs soon healed, and the check leaves remained free from spots.

Morphology

Vegetative cells.— The organism is a rather short rod, with rounded ends. It is found singly, in pairs, and, in liquid media, often in short chains. Individuals from a forty-eight-hours-slant Hiss-glucose culture are from 0.8 to 0.9 μ in length and from 0.3 to 0.5 μ in width. Bacteria taken directly from the twig cankers and stained with gentian violet measure from 1.6 to 1.8 μ in length and from 0.4 to 0.6 μ in width.

Endospores.— No endospores have been observed.

Flagella.— The organism is motile by means of one or several polar flagella. Both Pitfield's and Moore's flagella stains were used with good results. The flagella were also stained without a mordant, by exposing the covers to carbol fuchsin for from twenty-five to fifty minutes and carefully washing in alcohol (Fig. 67).

Capsules.— Capsules have frequently been stained in material obtained from Uschinsky's solution by both Welch's and Moore's methods.

Zooglœæ.— Zooglœæ are frequently found on various media.

Involution forms.— No involution forms have been observed.

Staining reactions.—The organism stains readily with the ordinary aqueous stains — aniline gentian violet, and carbol fuchsin.

Gram's stain.— Many efforts at staining were made with Gram's method, but the results were not sufficiently sharp and decisive to warrant a conclusion. In no case was the stain completely lost, nor was it ever retained in the original intensity. The aniline gentian violet and iodine solutions were used for two minutes and the preparations were left in absolute alcohol for three minutes. In no case were the mounts washed in alcohol as intensely stained as those not treated with alcohol, and consequently the organism must be regarded as Gram negative.

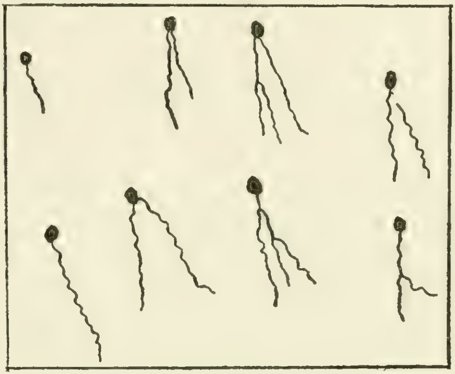

FIG. 67.—*Flagella of Bacterium Pruni. Stained from a culture of the peach strain of the organism grown in bouillon*

Acid-fast.— The organism is not acid-fast.

Cultural characters

Source of cultures.— Eighteen strains were used for each test, one from the apricot, one from the nectarine, nine from the peach, and seven from the plum. The reactions of all the strains on the various media were uniform, and no marked differences were observed except in one of the plum strains, which invariably grew most rapidly. The growth of this strain on potato plugs was more abundant than that of any of the other strains. Its growth on Hiss-glucose was often a number of hours in advance of the other cultures. Where there was any difference in the reaction one of the normal plum strains was always used as a basis for comparison.

Nutrient agar colonies.— When plantings were made from bouillon cultures six days old at room temperature of about 25° C., the colonies appeared as mere specks in from forty to forty-eight hours. Plantings made from diseased host tissue developed colonies in from three to ten days (usually from four to seven). No growth occurred at 37° C. The colonies were moist and glistening, and at first were almost white but later became amber yellow.[3] When not crowded, the surface colonies were round and reticulate, and reached a diameter of about one to five millimeters in ten days, with a smooth surface, an entire edge, and a finely granular internal structure. The deeper colonies were elliptical or lenticular. On plates twenty-five days old, colonies measured from ten to twenty-five millimeters in diameter. They had dark, translucent centers surrounded by a much lighter, often concentrically ringed, zone.

Nutrient agar streaks. On slanted agar the streak made with a platinum needle developed a moderate amber-white (4) growth. The streak widened slowly, chiefly toward the bottom, made a filiform growth, and was raised, with a slightly contoured surface and an entire edge; it was translucent, with a glistening luster, and was slightly viscid; it did not color the agar on which it grew, at least not for a number of weeks. Growth was moderate. After five or six days the growth began to flow downward, and finally accumulated in an amber yellow (1), slimy mass at the base of the slant.

Nutrient agar stab.— On agar stab the growth was filiform, and was best toward the top of the stab, there being very little or no growth in the lower half of the medium. A circular colony formed on the surface at the point of inoculation, and gradually spread, completely covering the surface in about seven or eight days with a straw yellow (2) growth. The agar was not liquefied nor softened.

Gelatin colonies.— On gelatin plates at room temperature of about 25° C., the colonies appeared as mere specks in from forty-five to fifty hours. They were at first almost white, translucent, moist, and glistening. On the fourth day they measured about one millimeter in diameter. Both buried and surface colonies were round, finely granular, and with an entire edge, becoming with age amber yellow (2) in color. Liquefaction began usually on the third day, the colonies becoming saucer-shaped, spreading, and liquefying the medium in twelve days or more.

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[3] All the color terms used in this paper are based on René Oberthür's *Répertoire de Couleurs*, published by La Société Française des Chrysanthémistes in 1905.

Gelatin streaks.— On slanted gelatin (+ 15) at room temperature of about 25° C., the streak having been made with a one-millimeter loop, a light, semi-transparent growth was noticed after two days along the entire path of the loop. In four days there was a slight liquefaction along the streak. On the fifth day the growth was amber yellow (1) in color, liquefaction had extended the entire length of the streak, and the growth was about ten millimeters wide and five deep. On the eighth day all material in the slant down to three millimeters below the base of the slant had been liquefied. Complete liquefaction occurred in from twelve to thirty days.

Gelatin stab. — In gelatin stab the path of the needle became beaded, but usually in the upper twenty-five millimeters of the medium it at first became more or less filiform. The growth on the surface was most rapid, and liquefaction, which began about the third day, soon passed to the walls of the tubes and then slowly downward. The growth in the depth of the gelatin soon ceased and the material along the track of the needle was not liquefied more rapidly than that away from its path. The liquefied gelatin was at first more or less clouded and was of about the same color as the solid material, but later it cleared and took on a raw sienna (2) color. A bacterial layer Naples yellow (3) in color soon formed on the solid gelatin and gradually settled as the material liquefied. Both a rim and a pellicle, cream yellow (2) in color, finally formed. Complete liquefaction occurred in from several days to six weeks or longer, depending on the temperature, the strain of the organism, and the composition of the culture material. In four months the liquefied material was viscid, was raw sienna (4) in color, and had a thick, slimy, Naples yellow (3), bacterial deposit in the bottom of the tube.

Hiss-glucose colonies.— Plantings made on Hiss-glucose from six-days-old bouillon cultures came up in from two to ten days (usually from four to seven) at room temperature of from 23° to 25° C., forming colonies from one to four millimeters in diameter in about eight days. No growth occurred at 37° C., but at 25° C. colonies appeared in forty-eight hours. The colonies were at first almost white, translucent, moist, and glistening, but later became amber yellow (1). When not crowded, the surface colonies were round, with an entire edge, the surface being smooth with a more or less convex elevation, and the internal structure being finely granular. The deeper colonies were elliptical or lenticular in form. Plates containing twenty cubic centimeters of the medium developed single surface colonies in thirty days which measured from sixteen to thirty millimeters in diameter. The older colonies were amber yellow (4) in color, were convex, showed a dark central nucleus, and were often concentrically ringed. The smaller, thin colonies had a semi-transparent luster.

Hiss-glucose streaks.— On Hiss-glucose slant-tube medium (+15), the streak having been made with a millimeter loop, growth was noticed in forty-eight hours at room temperature of about 25° C. A convex, slimy, straw yellow (2) growth developed along the path of the loop. The streak widened gradually (chiefly toward the bottom), soon became filiform, and in ten days completely covered the slant with a slimy yellow (2) growth. In culture three or four months old the medium was browned. Some of the growth soon moved downward and formed a slimy deposit at the base of the slant, which finally became amber yellow (4) in color. As the medium dried out the slimy deposit gradually settled, and in the five- and six-months-old cultures there was a slimy, amber yellow (4) bacterial deposit from five to ten millimeters deep in the bottom of the tube. Inoculated tubes placed in the incubator at about 37° C. also covered the slants in ten days, but the growth was of about the same color as the medium.

Hiss-glucose stab. - In Hiss-glucose stab the growth was filiform and best toward the top of the stab, there being very little or no growth in the lower part of the tube. A circular growth formed on the surface at the point of inoculation, and covered the surface in six or seven days. The medium was not liquefied nor softened.

Potato agar streaks.— In potato agar a slightly raised, wet, shining growth developed along the path of the needle in two or three days at room temperature of 25° C. The streak gradually widened, and finally covered about two-thirds of the surface of the slant, becoming amber yellow (1) in color. Some of the slimy growth soon moved downward and formed a deposit at the base of the slant. Growth was moderate, never completely covering the slant.

Dextrose litmus agar streak.— In dextrose litmus agar, there was slight growth along the path of the needle in two days. The streak was slightly elevated, and widened most rapidly at the bottom, becoming filiform. The slimy growth passed downward and accumulated at the base of the slant, and the medium below the mass became entirely bleached in five or six weeks.

Lactose litmus agar streak.— Growth in lactose litmus was not so marked as on the dextrose litmus medium. There was slight growth along the path of the needle by the close of the second day. The streak was slightly elevated, and it gradually widened and became filiform. The slimy growth passed downward and accumulated at the base of the slant. Growth was less abundant, and bleaching below the slimy mass only slight, at the end of six weeks.

Potato plugs.— Potato plugs were placed in running water for three hours, and were then put into tubes containing over five cubic centimeters of distilled water (the lower end of the plugs being in the water) and sterilized for twenty-five minutes at 100° C. for three consecutive days. The streak was made with a platinum needle from a six-days-old nutrient agar culture. The tubes were kept at room temperature of about 25° C. A slightly yellow, glistening streak developed along the path of the needle in two days. It made a moderate, slightly raised, echinulate growth, covering from one-half to two-thirds of the slant in about ten days but never completely covering it. One of the strains, however, repeatedly covered the entire surface of the cylinders in from five to six days. The growth had a contoured, wet, glistening, amber yellow (2) appearance. It was slimy, was free from odor, and turned the cylinder slightly gray. Most of the strains had only a slight action on potato starch.

Bouillon.— Both meat broth and meat extract bouillon were used. The meat broth material gave an acid reaction of + 15 Fuller's scale, and the meat extract bouillon recorded + 12. Inoculations were made from a three-days-old bouillon culture. All cultures were kept at about 25° C.

The medium was clouded after from twenty-four to forty-eight hours. An amber yellow (1) rim formed in from twelve to fifteen days, followed by formation of a pellicle. On being shaken, the material settled in the form of yellow, viscid flakes. The material cleared in about six or eight weeks and became raw sienna (3) in color, with a slimy, amber yellow (2) bacterial growth in the bottom of the tube. Very little difference was observed in the growths of the organism in extract bouillon and in broth bouillon.

Milk.— The milk used was maize yellow (1) in color, due to overheating in sterilizing, and was about 1.8 per cent acid. All tubes were inoculated with a one-millimeter loop of beef broth culture three days old, and kept at 25° C. Two days after inoculation there was a slight whey ring at the surface of the milk; after four days the ring was three or four millimeters deep. Smith (1903) states that this organism slowly precipitates casein, which is then gradually redissolved.

In about ten days the whey ring was five or six millimeters deep and was amber yellow (3) in color, and the fluid curd gradually changed to yellowish tan (4) in color. The amount of clear whey gradually increased, and in about twenty-five days it became gamboge yellow (2) and the curd gradually became sienna (4) in color. The whey was viscid, while the mobile precipitate finally became a compact, slimy mass. An amber yellow pellicle was formed in from fifteen to twenty days. Filamentary threads often extended from the pellicle to the curd below. The pellicle finally settled. On shaking tubes containing a well-formed pellicle, yellow, slimy threads sometimes extended from the surface of the medium to the bottom of the tube.

An amber yellow (2) layer of the organism soon formed on the curd, and gradually lowered in the tube as the curd dissolved. About the twenty-second day many white tyrosine crystals formed in this layer.

About the forty-fifth day the pellicle in many cases had settled. The whey now constituted three-fourths of the material, was viscid, and was saffron yellow (4) in color. The precipitate was a yellow-buff (1), slimy mass dotted with white tyrosine crystals.

In four-months-old cultures the material was about one-half dried out and the whey constituted about one-half of the material. It was Indian chestnut-red (2) in color, with a thick, slimy, fawn-colored (2) coagulum dotted with white tyrosine crystals.

Litmus milk.— In the test with litmus milk, the culture material had been incubated at 37° C. for forty-eight hours just before the inoculations were made. It was violet (4) in color, and a one-millimeter loop of a three-days-old beef broth culture was used for inoculating each tube.

On the second day a bluish violet (4) ring two millimeters deep had formed on the surface of the medium, and by the fourth day the surface ring was from six to eight millimeters deep, in many cases having an amber yellow (1) rim. The material below the bluish violet (4) ring still retained its original color, but gradually became dark violet.

In about fifteen days the top ring became pansy violet (4) in color and was from eight to ten millimeters wide. The amber yellow (4) rim was more prominent and usually a pellicle was formed. In some cases the deposit below the whey had changed to madder brown (2) in color. By the twenty-fifth day an amber yellow (1) pellicle had formed, in some cases with filamentary threads extending to the deposit below. On shaking of the tubes, the pellicle formed into thick, slimy threads sometimes extending from the surface of the medium to the bottom of the tube. In some cases the litmus was wholly reduced by the twenty-fifth day. The whey ring was about twenty-five millimeters deep, with the upper five millimeters pansy violet (3) and the lower twenty millimeters yellow lake (3) in color. The deposit in the bottom of the tube was brownish drab (2) in color, and was covered with an amber yellow (1) layer of the organism often three to four millimeters deep. Many white tyrosine crystals had formed in this layer. In about forty-five days the pellicle had settled, leaving a dull yellow-green (1) rim. The litmus had been in some cases completely, and in others only partially, reduced, but the material in most of the tubes had returned to a darker color. The whey by transmitted light was plum-violet (4) in color. There was a thick, slimy, brown-drab (2) deposit in the bottom of the tube, about 10 millimeters deep, and on the top of this an amber yellow (2) layer of the organism. Tyrosine crystals were scattered throughout the entire slimy mass.

In four-months-old cultures one-half of the material had dried out. The pellicle had settled, and a plum-violet (3) whey ring about twenty millimeters deep covered a thick, slimy, brownish drab (2) deposit six millimeters deep in the bottom of the tube. An amber yellow (2) layer of the organism on the top of the deposit was still rather prominent. Masses of white tyrosine crystals appeared throughout the entire slimy deposit.

Fermentation tubes.— A basal solution was made by adding two per cent of Witte's peptone to water. Six solutions were made from this, each containing one per cent, respectively, of one of the following carbon compounds: glycerin, saccharose, mannite, dextrose, maltose, lactose. Five fermentation tubes were filled with each of these solutions and sterilized by heating for twenty-five minutes on three successive days. Three tubes of each set were inoculated and two were left as a control. The inoculated tubes each received a one-millimeter loop of culture material from a five-days-old peach bouillon culture.

Five days after inoculation a cloudiness appeared in the open arm of each of the inoculated tubes. This turbidity increased with age, extending more than halfway into the U in the dextrose, saccharose, and maltose cultures; but at the end of twenty-five days there was no growth in the closed arm and no gas. There was a well-defined line between the growth in the bulb and the clear liquid in the closed arm, which seemed to point strongly toward the aërobic nature of the organism. A pellicle had formed on all the dextrose, maltose, and saccharose cultures, and in most cases there was a yellow, slimy growth of the organism at the bottom of the open arm. There was very little growth in the mannite tubes.

The cultures were tested for acidity after they had grown for twenty-five days. In glycerin the acidity increased from + 0.8 to + 1; in saccharose, from + 0.9 to + 1.1; in mannite, from + 0.9 to + 1; in dextrose, from + 1 to + 2.5; in maltose, from + 0.8 to + 1; in lactose, from + 0.8 to + 1.1.

Uschinsky's solution.— In the test with Uschinsky's solution, tubes were inoculated from a four-days-old broth culture. A one-millimeter loop of the material was placed in each tube.

The tubes were all clouded after three days. The solution often became viscid in about twenty-five days. A cream yellow (1) pellicle formed in from twenty to thirty days or later. The pellicle finally settled, forming a slimy, cream yellow (3) bacterial mass in the bottom of the tube.

Sodium chloride bouillon.— A one-millimeter loop of a six-days-old, + 12, peptonized beef broth culture was placed in tubes of peptonized beef broth containing, respectively, 1, 2, 3, 4, 5, 6, and 7 per cent of chemically pure sodium chloride. Three tubes were inoculated with cultures of each kind of host — apricot, nectarine, peach, and plum. At the end of seven days clouding was apparent in all tubes containing 1, 2, and 3 per cent of sodium chloride. Both the peach and the plum organisms grew in a 3.5-per-cent salt bouillon. There was no clouding in the 4-, 5-, 6-, or 7-per-cent solutions.

Growth in bouillon over chloroform.— By means of a sterile pipette, five cubic centimeters of chloroform was added to fifteen cubic centimeters of bouillon. Fifteen tubes were shaken twice a day during the period of this test. On the eighth day the tubes were divided into five lots, four of which were inoculated with six-days-old bouillon cultures of apricot, nectarine, peach, and plum organisms, respectively; the fifth lot being used as a control. These cultures were under observation for thirty-two days, but no growth was observed in any of the tubes.

Nitrogen nutrition.— In filtered river water containing one per cent of asparagin, the organism made a slow initial growth first visible in six days. When distilled water was used the first signs of growth failed to appear before the tenth day, and in some cases as late as the fifteenth day, even when the strongest, oldest cultures were used. A very slight growth was obtained in river water containing only dextrose. River water alone failed to give any growth.

Nitrates.— Twelve tubes, each containing ten cubic centimeters of peptonized beef broth to which had been added one per cent of nitrate of potash, were divided into four lots of three tubes each. The solutions in each lot were then inoculated with apricot, nectarine, peach, and plum strains, respectively.

At the end of ten days the tubes were clouded and a tube from each lot was tested for nitrates in the following manner: To each culture one cubic centimeter of boiled starch water and one cubic centimeter of potassium iodide solution (1 200) were added. A few drops of sulfuric acid were then introduced. The peach and plum cultures gave a slight blue reaction, and on the twentieth day the remaining tubes each gave a distinct reaction. However, six months later these experiments were again repeated three times, with the same strains of the four organisms, and not a single culture gave the reaction.

Indol.— A number of tubes of Uschinsky's solution with the addition of one per cent of Witte's peptone were inoculated with a five-days-old agar culture. Apricot, nectarine, peach, and plum strains were used in making these inoculations. The inoculated tubes all showed considerable growth on the fifth day, but a test for indol was not made until the tenth day. Sulfuric acid water (1–2) and dilute sodium nitrate (1–200 in water) were used. On the tenth day all cultures gave a slight rose color after being placed in boiling water for two minutes, indicating indol production. The other tubes were tested twenty days later and a well-marked indol reaction was obtained from all the cultures.

Cultures of the four strains made in Dunham's peptone solution gave positive results at the end of twenty days. There was a trace of pink before heating, and after four or five minutes in a water bath at 80° C. the reaction became distinct.

Toleration of sodium hydroxide.— Beef bouillon having a reaction of +10 Fuller's scale was used. Sufficient sodium hydroxide was added to obtain a reaction of 0, —2, —4, and so on for every two degrees of Fuller's scale to —30. The sodium hydroxide was added just before the last sterilization. Titrations made immediately following the sterilization, while the medium was still warm, showed an apparent loss of alkalinity. In many cases, although not always, the check lots of the medium titrated on the fifth day or later gave the original reaction.

Tubes inoculated and placed in an incubator at 25° C. gave a good growth in all cultures down to —10 in forty-eight hours. There was a slight growth in the —10 cultures, and at the end of two weeks a very slight growth occurred in the —13 cultures. No growth occurred in the tubes containing larger amounts of sodium. The best growth was in cultures containing from —2 to —4 sodium hydroxide.

Toleration of hydrochloric acid.— Ordinary beef bouillon having a reaction of + 12 Fuller's scale was used. Sufficient hydrochloric acid was added to 100 cubic centimeters of the bouillon to obtain a reaction of + 14, + 16, and so on for every two degrees of Fuller's scale to + 36. There was a very good growth in forty-eight hours in all the tubes up to + 22, and a slight growth in the lot having a reaction of + 24. A very slight growth was observed on the fourth day in the medium having a reaction of + 26; this growth became slightly stronger by the tenth day. No growth occurred in any medium containing larger quantities of acid. The best growth developed in lots containing from + 12 to + 20 acid.

Resistance to drying.— Small drops of four-days-old bouillon culture were placed on sterile cover glasses in a covered sterile petri dish, and left in an incubator at room temperature of from 18° to 23° C. At intervals of twenty-four hours one of the cover glasses was transferred to a tube of sterile bouillon. Three tubes were used for each test. Growth occurred up to the eighth day, after which the tubes remained sterile.

This experiment was repeated a number of times with bouillon cultures from four to ten days old. As a rule there was no growth after the eighth day; in one case, however, growth occurred on the fifteenth, and in another case on the twenty-first, day.

Effect of sunlight.— Hiss-glucose tubes inoculated from a three-days-old bouillon culture were poured into petri dishes and placed in the bright sunlight for forty-five and sixty minutes, respectively, half of each plate being covered with black paper. After six days, on each of six plates there were numerous colonies on the covered parts but none on the exposed parts. A few colonies, however, developed on the uncovered parts of all the plates exposed for fifteen, twenty, and thirty minutes.

Effect of germicides.— Poured Hiss-glucose plates were made with the plum organism after it had been exposed to various concentrations of copper sulfate, mercuric chloride, formaldehyde, lime-sulfur solution, the self-boiled lime-sulfur mixtures, and arsenate of lead.

In a copper sulfate solution (1–10,000), colonies appeared in five days on all plates on which cultures were made of the organism which had been exposed for one-half minute and for five minutes. However, there were only one-fourth as many colonies on the five-minutes exposures as on the one-half-minute exposures. No colonies appeared on any of the plates on which the organism had been exposed for fifteen minutes and for thirty minutes.

In a mercuric chloride solution (1–150,000), colonies appeared in five days on all plates on which cultures were made of the organism which had been exposed for one-half minute and for five minutes. The colonies on the plates exposed for five minutes remained small and failed to develop the yellow color. No colonies appeared on any of the plates on which the organism had been exposed for fifteen minutes and for thirty minutes.

In a formaldehyde solution (1–300), colonies appeared in five days on all plates on which cultures were made of the organism which had been exposed for one-half minute and for five minutes. However, there were three times as many colonies in the one-half-minute exposures as in the five-minutes exposures. No colonies appeared on any of the plates on which the organism had been exposed for fifteen minutes and for thirty minutes.

In a lime-sulfur solution (15–15–50 formula) in a 1–10,000 dilution, colonies appeared in five days on all plates on which cultures were made of the organism which had been exposed for one-half minute and for five minutes. However, there were only one-half as many colonies on the five-minutes exposures as on the one-half-minute exposures. No colonies appeared on any of the plates on which the organism had been exposed for fifteen minutes and for thirty minutes.

In a self-boiled lime-sulfur mixture (8–8–50 formula) in a 1–52 dilution, colonies appeared in five days on all plates on which cultures were made of the organism which had been exposed for one-half minute and for five minutes. No colonies appeared on any of the plates on which the organism had been exposed for fifteen minutes and for thirty minutes.

In arsenate of lead, 1–208 dilution, colonies appeared on all plates on which the organism had been exposed for one-half, five, fifteen, and thirty minutes, respectively.

Diastatic action.— The test for diastatic action was made on potato cylinders. Twenty-eight strains of the organism were grown on potato cylinders for twenty-five days. The cultures were then tested for starch reduction, and only one of the strains showed marked diastatic action. In all the other cases the only indications observed were a slow and slight tendency toward the conversion of starch into amylodextrin, shown by a gradual change in the iodine reaction from a clear blue to a purplish tint.

These tests were made according to the directions given by Harding and Morse (1909): "These potato cultures were crushed in porcelain mortars and 50 cubic centimeters of water added to separate the particles so that the effect of the iodine could be easily seen. The presence of unchanged starch was determined by the gradual addition of a weak solution of iodine in potassium iodide. After a sufficient amount has been added to satisfy some of the other compounds which are present the starch grains are turned to a blue-black. The extent of the reduction of the starch by the bacterial growth is estimated on the basis of similar tests of uninoculated potato cylinders."

The uninoculated cylinders, when tested with the iodine-potassium-iodide solution diluted with water, always yielded a bright blue reaction. The starch reaction was also strong after the organism had been grown on the cylinders for several weeks, although a purplish color indicated a slight diastatic action.

Fragments of potato scraped from under the yellow slime from a culture forty days old, mixed with about twenty times their bulk of water, gave a purple or a blue-purple reaction. When fragments of material from the back or the center of the cylinder were used and treated in the same way, they invariably gave the blue reaction. The water below the potato cylinder, when it contained more or less of the yellow slime of the organism, also invariably gave a purplish blue reaction.

Pepsin.— A peptonizing ferment must be present, since gelatin is liquefied and casein is slowly dissolved with the formation of tyrosine.

Lab ferment.— In the milk cultures the casein is thrown out of solution without marked acid production, which indicates the presence of a lab, or rennet, ferment.

Invertase.— Cultures of all four strains of the organism were grown in filtered river water containing two per cent of chemically pure saccharose and two per cent of Witte's peptone. On the twenty-fifth day all the cultures were tested for inverted sugar with Fehling's solution, and every culture gave the glucose reaction.

Thermal death point.— Preliminary tests indicated that the thermal death point must lie between 49° and 54° C. The following tests were made to determine the point more nearly: Six sets of twelve tubes each of peptonized beef broth were inoculated with the peach organism from a five-days-old-slant Hiss-glucose culture, and the tubes were placed in water at a constant temperature of 49°, 50°, 51°, 52°, 53°, and 54° C., respectively. The tubes were removed at the end of ten minutes, and were kept at 25° C. for two weeks. At the end of five days growth had taken place in all the tubes that had been exposed at 49° and 50° C. Growth appeared also in three of the twelve tubes that had been kept at 51° C. No growth appeared at the end of fourteen days in tubes that had been kept for ten minutes at 52°, 53°, and 54° C.

The experiment at 51° and 52° C. was repeated several times with different strains of the organism, and in a few instances growth occurred in cultures kept at 51° C. for ten minutes. There was never any growth in cultures kept at 52° C.

The thermal death point, therefore, is about 51° C. Smith (1903) also placed the thermal death point of this organism at about 51° C.

Optimum temperature.— The optimum temperature appears to lie between 24° and 28° C. Growth at 24° and at 28° C. on Hiss-glucose and peptonized beef bouillon was considerably better than at 32° C. Growth at 20° and at 36° C. was about the same, but was considerably less than at 32° C. and much less than at 24° or 28° C.

Maximum temperature.— Four-days-old bouillon cultures of ten strains of the organism were used to inoculate slant Hiss-glucose medium. Six tubes were used for each strain; three tubes of each lot were placed in an incubator at 37° C., and three were kept at room temperature. All cultures, both in the incubator and at room temperature, made good growth; however, those exposed to the higher temperature were of the color of the medium, while those in the lower temperature had developed the normal yellow color. On the fifteenth day the heat of the incubator was reduced to 25° C.; on the sixteenth day one of the cultures had taker on a yellow color, and by the eighteenth day all the cultures had developed the normal color. From these results it is evident that 37° C. is close to the limit of growth for the organism on Hiss-glucose.

In a series of experiments, slant Hiss-glucose and bouillon cultures were kept at 20°, 24°, 28°, 32°, 36°, and 40° C., respectively, for three weeks. Six-days-old bouillon cultures of four strains of the organism were used for making the inoculations. Two three-millimeter loops of the fluid were used for each tube. Three tubes of bouillon and two of the slant Hiss-glucose medium were used in each set. Five tubes of bouillon and three of the Hiss-glucose medium were also inoculated and were placed at room temperature (from 23° to 25° C.), to serve as checks. All strains of the organism on both kinds of media made decidedly the best growth at 24° and at 28° C. They also made better growth in both media at 20° than at 32° C., and the growth at 32° was considerably better than at 36° C. There was no growth at 40° C.

The growth on Hiss-glucose cultures kept at 20° and at 24° C. developed the normal yellow color; tubes kept at 28° C. developed a growth a shade lighter in color; those kept at 32° C. were much lighter, and had a mere trace of yellow; in those kept at 36° C. the yellow shade had disappeared entirely, and the growth was of the color of the medium.

Loss of virulence.— Park (1905) states that " bacteria differ . . . as to the ease and rapidity with which they grow in any nutritive substance and the amount of poison they produce. Both of these properties not only vary greatly in different members of the same species, but each variety of bacteria may to a large extent be increased or diminished in virulence."

In June, 1910, four cultures, one each from apricot, nectarine, peach, and plum twigs, were obtained at Mountain Grove, Missouri. All the cultures were grown continuously on a Hiss-glucose medium. It was observed that the plum organism obtained at this time was the most vigorously growing strain in the laboratory. In the inoculation work in Missouri during 1910 it was also the most aggressive on all four hosts. In the work at Cornell University during 1911 the writer was much surprised to find that not only had this strain lost its vigor on the various culture media, but the injury was also far less marked when it was inoculated on the green tissues of the different hosts. In the fall of 1911 Abundance plum twigs were inoculated with cultures of this strain, and in the summer of 1912 the organism was regained from one of the twigs. The culture was again grown continuously on a Hiss-glucose medium. The regained culture made a slightly better growth on the various media than did the culture which had been growing continuously on the Hiss-glucose medium, and was also slightly more aggressive when inoculated on the green tissues; but it was far less virulent than when first obtained. In the summer of 1912 the four old strains which had been growing continuously on the Hiss-glucose medium since 1910 were rejuvenated by growing them in bouillon for fifteen days, making transfers to a new medium every third day, and finally on the fifteenth day all were again placed on the Hiss-glucose medium. The plum organism at once became again the most vigorous grower, and when inoculated on peach leaves and twigs it was also the most aggressive. From these results it appears that vigor is the controlling factor in the virulence of the organism.

Group number.— The group number, according to the descriptive chart of the Society of American Bacteriologists, is 211.2232523.

Life history

Inoculation.— *Bacterium Pruni*, so far as is known, passes its entire life cycle in nature within the tissue of the host. The disease usually makes its appearance in the spring shortly after the leaves have unfolded, but as a rule serious outbreaks do not occur before May and often not until later. Cankers on the twigs and limbs are the principal sources of infection in the spring. Trees bearing twigs on which inoculations result in the development of well-formed cankers, are invariably the first to develop leaf spot abundantly the following year. Young trees which suffer severely from repeated outbreaks of the shot-hole condition usually show many twig cankers. Twig cankers are also constantly associated with the early spring outbreaks on older trees. The tissues of the bud may likewise become invaded, and are often responsible for the early spring infection. Eleven out of fifty cultures made from the centers of buds taken in August from peach twigs on which the foliage was badly shot-holed, yielded many colonies of the yellow organism.

The bud cut from bud wood obtained from an infected tree often conveys the organism to healthy stock. Laboratory experiments indicate that when exposed to sun and air the bacteria which lodge on twigs and buds retain their vitality for only a very short time.

If the twigs are killed after the cankers are well formed, the organism may remain alive in the dead tissue for a considerable time. Many peach and plum twigs cross-inoculated by the writer in the summer of 1910 died in the following winter, but cultures of the organism were repeatedly obtained from the dead twigs up to the middle of the next June. From that time on it was impossible to secure cultures from this source. Cultures were readily obtained, however, from the cankers on the green inoculated twigs.

Old leaves on the ground, if well protected from sun and air, may possibly harbor the organism for some time, but it is doubtful whether they ever constitute an important source of infection. It is possible that pruned twigs and limbs left on the ground may occasionally be the source of primary infection, but in the large percentage of cases the primary infection can be traced to cankers on twigs and limbs of the trees. The writer's experiments indicate that the organism soon loses its vitality in material on the ground.

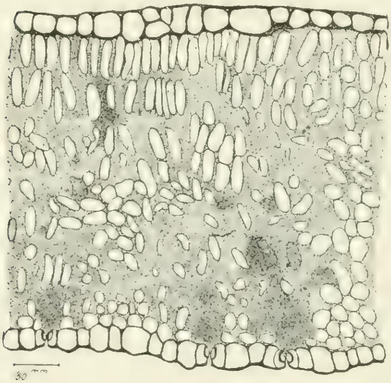

Fig. 68.—*Cross section of a peach leaf which was inoculated with a strain of Bacterium Pruni from the fruit of the plum. The material was collected eight days after the inoculation had been made. The organism entered the leaf through the stomata.* (*Drawing made with the aid of a camera lucida*)

In October, 1911, fifteen hundred peach leaves, one thousand plum leaves, and fifty peach twigs, which had been cross-inoculated with cultures of the four strains of the organisms, were put between wire screens and left over winter on the ground, where they were fully exposed to the sun. In April, 1912, all attempts to regain the organism from these twigs and leaves failed. In another experiment, however, Hiss-glucose poured-plate cultures made on June 1, 1913, from diseased leaves that had been collected on October 10, 1912, and kept in the laboratory between blotters, yielded colonies of the organism.

The organism gains entrance to the host through the stomata (Figs. 68 and 69) and the young lenticels during periods of sufficient moisture. A film of water on the surface of the leaf, the twig, or the fruit enables the bacteria to move about and gain entrance to the substomatal cavity. Inoculation ordinarily does not take place in the absence of moisture. Lenticels and stomata are fairly well distributed on the young twigs, and the stomata are more or less plentiful on the green fruit and on the lower surface of the leaves, but there are none on the upper leaf surface. Consequently inoculation may occur anywhere on the young, tender twigs and the green fruit, while on the leaves the organism can gain entrance only from the lower side.

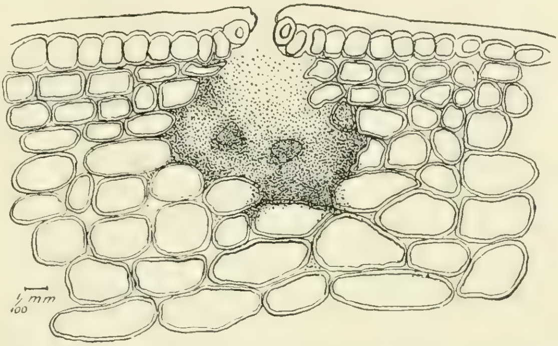

Fig. 69.—*Early stage of fruit spot on Burbank plum fruit. The fruit had been inoculated with a strain of Bacterium Pruni, isolated from a peach tree. The material was collected seven days after the inoculation was made. It was fixed in strong alcohol, infiltrated with paraffin, and cut on the microtome. The sections were stained with acid fuchsin. (Drawing outlined with the aid of a camera lucida)*

In 1911, at Ithaca, New York, 332 Elberta peach leaves of different ages were inoculated in the early morning. A few drops of water containing numerous bacteria in suspension were placed, by means of a pipette dropper, on the upper or the lower surface of each leaf. In all, 149 of the leaves were inoculated on the upper, and 183 on the lower, surface. In four weeks 177 of the leaves inoculated on the lower surface contained many spots; 6 were clean. All the 149 leaves inoculated on the upper surface were free from infection. In the same year 100 plum leaves were inoculated in the same way, 40 on the upper and 60 on the lower surface. At the end of five weeks, 55 of the leaves inoculated on the lower surface contained many brown spots, and 5 were free from lesions. All the leaves inoculated on the upper surface were free from spots. All the check leaves were normal.

From the results it was evident that a moist lower surface is essential for inoculation and spread of the pathogen on the leaves. Observations show that dew plays quite as important a part in the dissemination of the pathogen as does rain. Rain and dew are not only important factors for inoculation, but they also carry the bacteria to the healthy leaves, twigs, and fruit, and thus frequently serve as agents of transportation.

The bacteria are no doubt carried from tree to tree and disseminated on the young leaves, fruit, and growing twigs by insects of various kinds. Waite (1898), Stewart (1913), and others have proved that bees, wasps, flies, snails, aphids, and beetles carry the pear blight organism from oozing cankers to open blossoms and young shoots, and thus spread the blossom and twig blight of apples and pears throughout the orchard. From the large numbers of various insects often found working on the diseased foliage and fruit of trees affected with *Bacterium Pruni*, and from the nature of the bacterial ooze, there appears little doubt as to the importance of insects in the dissemination of this organism.

In spring, when the weather is cold and unfavorable for the development of the organism and the insects are sluggish, only a few primary inoculations occur and only a few scattered spots develop on the foliage. As the season advances and the weather becomes warmer, the bacteria multiply and escape from the spots of the early infections, the secondary infections gradually become abundant, and the leaves that contained only a few spots at first soon become dotted with them. Insects also become more active, and secondary spots of infection multiply rapidly.

In the inoculation experiments on plum leaves in 1913, snails and aphids were undoubtedly the chief agents in conveying the organism from the inoculated diseased leaves to the young leaves as they unfolded. The new leaves of the trees on which there were no snails nor aphids remained free from infection. Poured plate cultures were made from each of eleven snails and eighteen aphids collected from these trees. Three of the snail cultures and two of the aphid cultures yielded colonies of the organism.

The great frequency with which infection occurs on the leaves along the path taken by ants leaves little doubt that these insects also aid more or less in spreading the disease. Nevertheless, cultures from more than one hundred ants gave negative results.

As a rule, insects merely carry the virus from leaf to leaf or from tree to tree, and seldom introduce the bacteria directly into the tissue of the host.

Still the writer has frequently obtained cultures of the organism from both feeding and egg punctures of curculio on fruit, and in a number of cases it has been practically certain that the insect was the agent of inoculation.

Infected leaves are often blown about by the wind, and no doubt are sometimes responsible for the occurrence of primary infection. Yet danger from this source is comparatively slight, since the leaves soon dry out and the bacteria quickly lose their vitality. The disease usually advances in the orchard in all directions from a center of infection, often forming a more or less circular diseased area. After the organism has become established, the movement of limbs and branches caused by high wind during storms also furnishes a means of conveyance for the bacteria from diseased to healthy organs.

Old neglected trees, the plum in particular, often serve as sources of inoculum. In the past seven years two outbreaks of the disease on the Missouri State Fruit Experiment Station grounds were traced directly to infected Burbank, Abundance, and Golden plum trees.

Where the organism has become well established, diseased trees may serve as a source of inoculum and young trees planted in adjoining fields gradually become diseased. In 1906 a very striking case of this sort came under observation. A tract of one hundred and sixty acres of land on the east side of an old peach orchard at Koshkonong, Missouri, which contained many infected trees, was planted in the spring with two-years-old Elberta peach trees. In October the foliage and twigs of the trees in the ten rows next to the old trees were badly diseased. During 1907 the organism gradually advanced, and by the close of the season the trees in one hundred and ten rows were infected. The disease, however, was much worse in the first thirty rows than in the others. During 1908 the organism spread entirely across the orchard and every tree in the entire tract was injured to a greater or less extent. The trees in the first thirty rows next to the old orchard suffered most severely, and many of them were permanently injured.

Man himself is often an important agent of inoculation. In selecting material for budding, nurserymen frequently cut twigs from diseased trees, and thus, by inserting the infected bud material, inoculate the stock.

In 1907, on the Missouri State Fruit Experiment Station grounds, several thousand healthy peach seedlings were budded with buds taken from infected Elberta twigs. During 1908 the shot-hole condition developed on the foliage of nearly all these trees, and cankers were more or less plentiful on the twigs of many of them. Trees of this sort, when set out, not only grow poorly, but also serve as centers from which all neighboring trees become inoculated. In the course of the past five years several cases have come under observation in which a few infected trees from a poorly kept nursery were mixed in large shipments of clean trees. The infected trees served after planting as inoculum centers, and three years after planting every tree in these orchards was diseased.

In a careful inspection of peach and plum trees in various sections of South Carolina, not a single case of the disease was observed on isolated seedling peach and plum trees; yet the organism was repeatedly collected on the budded peach and Japanese varieties of plum in both family and commercial orchards, indicating that the nurseries are largely responsible for the spread of the pathogen.

Incubation.—The period of incubation varies more or less, depending on temperature, moisture, and method of inoculation. The period is about the same on all four kinds of trees. In some cases, however, it appears to be slightly longer on leaves and twigs of apricot and plum than on those of the nectarine and the peach.

In experiments in which the organism was suspended in water and applied to the leaves and fruit with an atomizer in warm, moist weather, the period apparently varied from seven to fifteen days, but in cold weather the period was prolonged to twenty and even twenty-five days. When water containing the organism was applied in warm weather to the lower surface of the leaf and to the fruit by means of a pipette dropper, and was spread by gently rubbing leaf or fruit between the thumb and the fingers, signs of disease appeared on the leaves in from five to seven days and on the fruit after seven or eight days; in cold weather, however, the period was considerably extended on both leaves and fruit. On the twigs, considerable difficulty having been experienced in obtaining results with the spray and rubbing method, a hypodermic syringe was used. The needle was inserted just beneath the cuticle and a small quantity of water containing the bacteria was forced into the tissue. Very uniform results were obtained in this way. The period of incubation following these inoculations varied from four to ten days, depending largely on the temperature and on the number of organisms forced into the tissue. In the twig experiments moisture did not influence the period of incubation to so marked an extent as in the experiments with leaves and fruit.

Infection.—Infection occurs soon after the bacteria pass through the stomata into the substomatal chambers, where they multiply and secrete a cytolytic enzyme which soon breaks down the cell walls of the neighboring cells, giving the organism access to an abundant food supply.

Serial sections made from infected plum fruit show that the organism at first occupies merely the substomatal chamber, but the neighboring cells are soon broken down, and as the bacteria multiply, the deeper tissues become involved. In about six or seven days a small cavity filled with bacteria is formed, and the first evidence of disease appears in the form of a water-soaked spot just beneath the stomata. A cross section through one of these spots shows the cavity filled with a compact mass of bacteria (Fig. 69, page 415). In about ten days or more, depending much on the weather conditions, the bacteria escape to the surface through the stomata or a slight central rupture. In some cases the bacteria burrow deeper and spread laterally, forming a somewhat circular, sunken, black area. The bacteria reach the surface through the various stoma and central rifts.

The tissues of the leaf are invaded in much the same way as are those of the fruit. At first the parenchyma tissues are those principally involved, but finally the vascular bundles also may be attacked (Fig. 68, page 414). The external signs of injury sometimes appear a day or two earlier on the leaves than on the fruit, and the ooze may also be evident a little earlier. The area in which the cells have been broken down assumes a more or less circular form and soon becomes separated by a line of cleavage from the healthy tissue. The injured tissue within this area soon contracts, dries out, and falls away, leaving a fairly clean-cut hole in the green tissue.

The tender tissues of the young shoots are also injured in much the same way, but the incubation period in the older tissues of the twigs is apparently longer and the development of the water-soaked spots is often slower. A cross section through one of these spots also shows a cavity filled with a compact mass of bacteria. The black-spot condition on the twig appears in from twenty to thirty-five days. Many of the phloëm cells of the sunken black area are broken down and the entire tissue is more or less disorganized (Fig. 70). Radial rifts or pockets in the young wood and cortex extend outward, often to the epidermis. As the twigs increase in size these rifts open and form an approximately longitudinal fissure. The bacteria escape through these fissures in countless numbers as bacterial ooze.

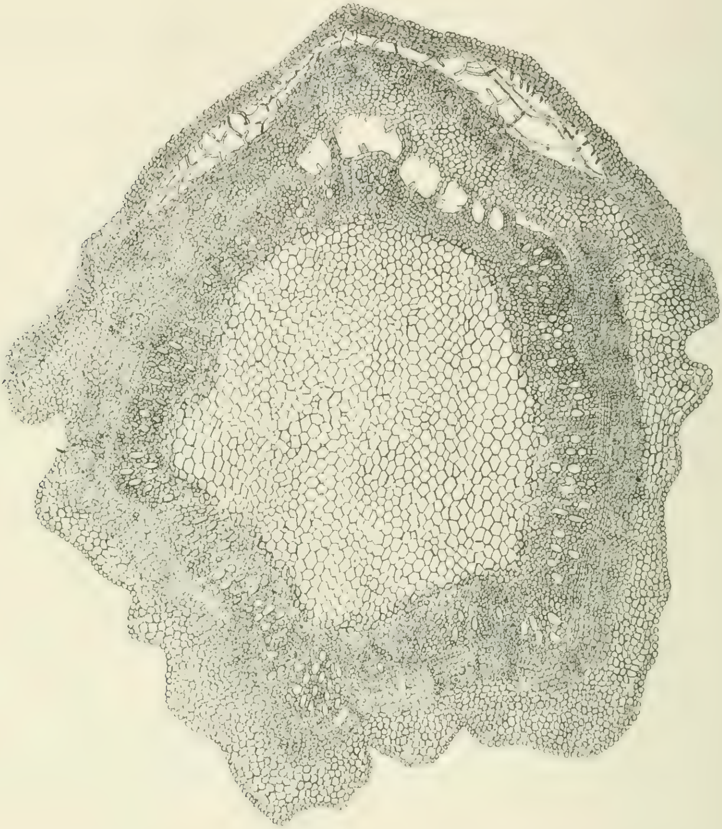

FIG. 70. — *Cross section of a young peach twig inoculated with a pure culture of Bacterium Pruni obtained from a plum twig. Bacterial pockets are most abundant in the cortical parenchyma. All cortical parenchyma cells in the infected region are distorted. The bacterial pockets in the cambium region also are to be noted. Occasionally the large ducts in the young wood are filled with the organism*

New infections occur only on the current season's growth. The organism is most aggressive in the young, tender tissues of the twigs. As the tissues harden, the influence of the organism in many cases becomes less marked and the blackened areas gradually become lighter in color; by the close of the season these areas have in many cases assumed much the same color as the old bark. During the second year a large proportion of the twig cankers thus disappear. A cross section of a twig through such cankers shows that no new bacterial pockets have appeared near the cambium zone and the old cavities have gradually been crowded into the outer bark. The organism, however, in some cases at least, is still alive and active, and no doubt often serves as a source of inoculum.

EFFECT OF ENVIRONMENTAL FACTORS

The condition of the weather is the most important environmental factor with respect to this disease. This is particularly true as regards moisture, but temperature is also of marked consequence. The shot-hole condition does not usually appear until about the middle of May or the first of June. In a very warm, damp spring, however, it may appear as early as the first of April, while in other years severe outbreaks may not appear until in August or even later. A temperature of from 20° to 28° C. is the best for the growth of the bacterium. Cold weather not only retards its growth, but also checks the activities of insects, which are the important agents in disseminating the organism. In the writer's spring and fall field work, it was frequently observed that when a cold period followed immediately after a series of inoculations had been made, the growth of the organism was retarded, and in some cases three, and even four, weeks passed before signs of disease appeared. The warm, slow, continued rains of the summer furnish the best conditions for the rapid spread of the disease. Heavy, driving rains of short duration followed by sunshine and winds are not favorable to its spread, since many of the bacteria are washed to the ground, and the leaves are quickly dried off and the few bacteria that may be spread will be quickly dried and killed.

The foliage of eight three-years-old peach trees, which were growing in boxes in the open, was inoculated with pure cultures of the organism. In three months the foliage on all the trees was dotted with brown spots. Four of the trees were then moved into the greenhouse and water was applied only to the roots. No new spots appeared on the old leaves after the second week, and all the new leaves that developed in the greenhouse remained free from infection, while the leaves of the four trees in the open continued to develop many new spots.

The relation of moisture to the spread of the disease is also brought out to a marked extent in the field when a favorable wet period is followed by a dry period. All the leaves formed in wet weather contain many brown spots and usually develop the shot-hole condition more or less, while those formed in a dry period remain free from spots, the contrasts in the foliage of the two periods being sometimes very striking.

Heavy dews, if the temperature does not fall too low, also furnish favorable conditions for inoculations, and observations indicate that they often play quite as important a part in the progress of the disease as does rain.

Shaded situations are favorable for the growth of the organism. Direct sunlight soon kills it. However, since the stomata of the leaves occur only on the lower surface, and the twigs and the fruit are usually fairly well shaded by the leaves, only comparatively few of the organisms ever become exposed to the direct rays of the sun.

In dry seasons injury from this organism is very slight, especially if the spring has been cold. Infections in such seasons are found only in the center of the tree, where the foliage remains moist from the dew until late in the day.

While it is true that temperature and moisture are the factors that govern the distribution of the organism, yet soil conditions, fertilizers, and cultivation have a marked influence on the extent of injury done. The soil in which the trees are growing has a considerable influence on the development of the disease. When a general outbreak occurs in an orchard, the trees on the higher, thin ridges invariably suffer more than those on the lower, more fertile land. Trees that have borne the heaviest crops in previous years invariably suffer more than do those that have yielded little or no fruit in previous seasons. Young trees planted in new land properly broken up before planting are more nearly free from the disease than trees planted on land poorly prepared. Trees properly pruned and well cultivated show less disease than do those that are not pruned nor cultivated.

VARIETAL SUSCEPTIBILITY

Observations show that the different varieties of the various hosts exhibit considerable variation in their susceptibility to the disease. The plums show the greatest variation. The majority of the American varieties suffer little, if at all, while nearly all the Japanese varieties, especially Abundance and Burbank, are severely injured, in some cases the disease resulting in the formation of deep limb and trunk cankers and in eventual death of the tree. Similar wounds are occasionally formed also on some varieties of apricot, nectarine, and peach, but they are far more abundant on Japanese varieties of plum.

The fruits of different varieties of the four kinds of hosts also show considerable variation in susceptibility. The nectarines suffer the greatest injury, the fruits of all the varieties of this host in Missouri being so badly cracked that they are usually destroyed by the various rot-producing organisms while still green. The effects of the disease on the fruits of Japanese plums, especially Abundance and Burbank, are nearly as severe as on the nectarines. The greater proportion of the fruits are badly distorted and cracked and the small number that are not rotted before they ripen are so badly injured that they are worthless for market. As a rule, apricot fruits are injured to a less extent than those of the nectarine or plum, but the variety Royal is severely affected. Though the fruits of some varieties of peaches, particularly Elberta, are severely injured, they are as a rule less badly disfigured than are the fruits of the nectarine or the Japanese varieties of plum (Fig. 63c).

In addition to this variation in susceptibility of the different varieties, there is also considerable variation in the amount of injury to the fruits of a given variety during different seasons. The real significance of these latter variations is not always easy to explain, but in most cases the age of the fruit and the weather conditions determine largely the percentage of infection.

In table 1 is given a list of the apricot, nectarine, peach, and plum trees in the variety orchard of the Missouri State Fruit Experiment Station, showing the nature and degree of susceptibility of the different varieties. These figures represent the average record for the year 1909. An estimate of the percentage of infection on leaves, twigs, and fruit was made by passing through the orchard, estimating and recording the injury. After the estimates had been made several trees in different parts of the orchard were carefully examined and the actual percentage of infection was obtained by counting the diseased leaves, twigs, and fruits. The difference found between the actual and the estimated percentages of infection formed the basis for correction of all the varietal estimates. Three estimates were made in the course of the summer, the first in the middle of July, the second August 5 to 7, and the third September 26 to 29. The average result of the three records is given here as the record for the year.

TABLE 1. RELATIVE SUSCEPTIBILITY OF DIFFERENT VARIETIES OF APRICOT, NECTARINE, PEACH, AND PLUM TO BACTERIUM PRUNI*

Apricot

Bread, 40-30-30-f
Moorpark, 40–20–0–f
Peach, 75–30–0–a
Russian, 30–10–?–a
Superb, 20–15–0–p
Sweet Russian, 40–20–30–p

Nectarine

Coosa, 45–15–100–a
Munson's Cling, 50–8–85–a
New White, 58–8–100–p
Stanwick, 65–15–100–a
Victoria, 60–10–100–a

Peach

Alexander, 25–15–5–p
Alice Hampton, 20–15–35–p
Alton, 40–20–80–a
Amsden June, 8–5–25–f
Arkansas Beauty, 35–15–50–a
Arkansas Mammoth, 50–10–50–f
Arkansas Traveler, 40–10–0–a
Arnett, 55–10–?–p
Arnett No. 6, 65–5–?–a
Athens, 40–3–30–a
Banner, 15–8–35–n
Barber, 8–5–0–f
Barnard Early, 35–10–60–a
Barnes, 5–3–0–f
Beauty, 30–10–50–a
Belle (Belle of Georgia), 15–3–25–a
Bell's October, 20–10–25–n
Bequette Cling, 23–3–45–p
Bequette Free, 40–10–70–a
Bernice, 35–10–25–f
Bilyeu, 60–10–100–a
Bishop, 18–18–0–f
Blanchard, 55–10–45–a
Blood Cling, 45–10–0–p
Blood Free, 50–10–30–p
Blood Leaf, 55–5–45–a
Bokhara, 0–10–40–n
Bonanza, 45–15–60–p
Brandywine, 6–13–40–f
Bray's Rareripe, 25–15–30–f
Brett (Mrs. Brett), 20–30–30–a
Brigdon, 40–10–20–a
Briggs (Briggs' May), 18–18–40–f
Bronson, 30–15–25–a
Burke, 20–10–35–p
Carman, 60–0–30–p
Carpenter (Carpenter Cling), 35–5–50–a
Chairs (Chairs' Choice), 30–20–35–a
Champion, 60–0–30–p
Champion Yellow, 15–5–30–f
Charlotte (Early Charlotte), 35–10–?–p
Cherokee, 25–20–45–a
Chili (Hills' Chili), 20–10–30–p
Chilow, 25–40–25–p
Chinese Cling, 45–15–30–a
Chinese Free, 45–5–55–a
Christiana, 30–5–25–a
Cloves, 43–3–50–p
Cobler, 25–15–20–p
Coleman, 20–10–30–p
Collridge Favorite, 30–10–30–a
Columbia, 35–15–40–a

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*The first number following the name of the variety indicates the percentage of infected leaves; the second, the percentage of defoliation; the third, the percentage of infected fruit (" ? " in the third place indicates that the tree bore no fruit). The letter in the fourth place indicates the number of twig cankers observed: a, abundant; p, plentiful; f, few; n, none. The letter in the fifth place (in the plum varieties) indicates the amount of injury due to trunk and limb cankers: e, extreme injury causing the death of the trees; m, marked injury; s, slight injury; n, no injury.

Peach (*continued*)

Conkling, 13–8–0–f
Connecticut, 43–3–60–p
Connet (Connet's Early), 15–10–60–f
Cora, 80–8–10–a
Crosby, 13–3–0–f
Crothers, 60–0–100–a
Davenshire, 40–20–0–a
Davidson, 35–20–50–p
Dawson's Early, 25–2–0–f
Delaware, 45–5–60–f
Deming (Deming's September), 60–10–40–a
Denton, 55–15–45–a
Dewey (Admiral Dewey), 20–10–0–p
Diamond, 50–20–30–a
Dover, 45–5–70–a
Early Barnard, 10–20–0–n
Early Crawford, 10–5–25–f
Early Heath, 35–5–25–p
Early Michigan, 30–5–80–a
Early Rivers, 30–15–40–f
Early York, 40–10–50–a
Ede (Captain Ede), 15–10–25–p
Edgemont Beauty, 11–5–25–p
Elberta, 80–30–45–a
Elberta Cling, 60–10–53–a
Eldred, 65–15–35–a
Emma, 50–15–50–a
Engle (Engle's Mammoth), 15–10–0–f
Etna, 20–10–25–p
Eureka, 45–10–55–a
Family Favorite, 80–10–70–p
Fischer, 65–5–100–p
Fitzgerald, 35–10–35–a
Flatters St. John, 65–5–80–a
Flemer's Cling, 65–5–98–a
Flewellen, 35–5–33–a
Floyd, 50–10–40–a
Ford's No. 1, 50–0–70–p
Ford's White, 40–10–25–f
Foster, 30–20–30–p
Fox (Fox Seedling), 18–8–35–a
Frances, 30–10–50–a
Froth's Early, 65–15–55–a
Future Great, 20–10–25–p
Garfield, 13–8–35–p
Geary's Hold-on, 28–8–25–p
General Taylor, 5–5–95–p
Gilman's Favorite, 50–10–45–a
Globe, 15–10–50–p
Gold Drop, 21–10–60–p
Gold Dust, 30–75–30–p
Golden Gate, 50–20–?–a
Gold Medal, 40–10–30–a
Gold Mine, 30–10–35–p
Gordon, 20–15–30–f
Grand Reporter, 55–15–50–a
Hale Early, 38–8–40–p
Heand's Beauty, 60–10–45–a
Heath (Heath Cling), 60–20–60–a
Heath Free, 60–20–25–a
Henrietta, 8–5–40–f
Hiley, 26–1–40–p
Hill's Madeira, 25–5–40–p
Hobb's Late, 15–10–22–f
Hobson, 30–25–?–a
Holderbaum, 20–5–25–f
Honi Chirf, 35–10–30–p
Hoover's Heath, 20–10–10–n
Horton's River, 45–15–70–p
Hughes I. X. L., 30–10–0–p
Hyde's Yellow, 20–5–20–f
Hynes (Hynes' Surprise), 20–10–30–f
Indian Blood Cling, 10–10–0–n
Indian Blood Free, 50–10–40–a
Ingold (Lady Ingold), 30–20–90–a
Japan Blood, 55–5–0–a
Jaques (Jacques' Rareripe), 15–15–25–f
Jennie Worthern, 35–10–30–a
Juno, 45–5–30–a
Kalamazoo, 10–8–0–f
Kerr (Jessie Kerr), 25–10–0–f
Krummel's October, 20–10–45–p
Late Crawford, 40–10–40–a
Late Elberta, 60–15–35–a
Laure Cling, 35–5–30–a
Lemon Cling, 12–10–0–f
Lewis, 0–10–50–n
Lodge, 40–10–60–a
Lola (Miss Lola), 25–10–40–f
Lorentz, 33–8–0–p
Louise (Early Louise), 35–15–0–a
Lovell No. 3, 50–0–?–a
McCallister, 20–15–?–f
McIntosh, 13–8–0–p
Madison County Mammoth, 40–10–70–a
Magnum Bonum, 35–30–25–a
Mamie Ross, 20–10–40–a
Mammoth Cling, 75–15–50–a
Marshall, 30–10–30–p
Mathews (Mathews' Beauty), 50–20–50–a
Mena, 40–10–?–a
Michigan, 10–5–0–n
Mid September, 23–8–25–p
Mint's Free, 30–20–80–p
Mixon Late, 75–5–30–a
Moore's Favorite, 20–15–50–a
Morris Late White, 50–10–30–p
Mountain Rose, 25–15–70–p

Peach (*concluded*)

Muir, 25–15–30–a
Munson's Cling, 65–15–100–a
Munson's Free, 60–10–70–a
Nanticoke, 23–8–30–f
Nelson's No. 5, 40–10–45–a
New Prolific, 10–15–25–f
Niagara, 45–5–40–p
Nicholson's Smock, 40–10–40–p
October Free, 50–10–33–a
Olden No. 1, 40–10–25–p
Olden No. 3, 50–10–100–a
Oldmixon Cling, 55–15–30–a
Oldmixon Free, 50–20–35–a
Oldmixon Improved, 30–15–45–p
Orange Cling, 40–20–35–p
Oriole, 30–3–55–p
Oritz, 28–18–40–a
Oscar, 63–8–70–a
Ozark Queen, 20–20–40–p
Pallas, 43–3–70–a
Pearce's Yellow, 30–10–0–p
Peento, 5–5–?–n
Pendleton, 40–10–25–a
Perry's Red Cling, 35–15–?–p
Plaut, 50–10–35–a
Pond's Seedling, 70–10–40–a
Pool's Favorite, 8–3–0–f
Queen, 40–20–50–p
Rasin Cling, 35–25–60–p
Ray, 20–5–30–p
Red Heath, 35–5–25–a
Red River, 60–15–30–a
Reeves Yellow, 20–15–0–f
Reynolds, 18–8–25–f
Ringgold, 40–10–40–p
Rivers, 58–8–25–a
Robert, 50–20–30–a
Rodgers, 20–5–45–p
Rose of Baltimore, 48–8–30–a
Rush's Favorite, 30–10–65–a
Russell, 23–20–20–n
St. John, 50–20–80–a
Salway, 15–15–35–f
Schofield, 60–0–40–a
Scott's Nonpareil, 65–15–40–a
Sea Eagle, 30–10–30–f
Sea Eagle Improved, 25–10–30–f
Shipler, 42–15–60–a
Shoemaker, 20–2–0–n
Smock (Smock's Free), 50–20–50–a
Sneed, 70–25–40–a
Snow, 35–10–25–a
Snow Free, 30–5–45–p
Snow's Orange, 23–10–0–n
Springfield, 50–10–40–p
Steadley, 30–10–35–p
Stevens, 60–10–35–a
Stevens Late, 20–20–40–f
Stinson, 65–15–65–a
Strout's Early, 15–10–?–f
Stump, 15–10–40–p
Success, 30–10–20–p
Summer Snow Cling, 40–10–55–a
Superb, 40–20–40–p
Sussex's Late, 35–5–45–p
Sylphide, 33–13–23–p
Texas (Texas King), 10–5–65–f
Thurber, 15–3–30–f
Tillotson (Early Tillotson), 10–12–0–n
Toledo (Early Toledo), 35–5–0–p
Topaz, 15–15–50–a
Tornado, 30–10–60–p
Trebout, 50–20–70–a
Van Buren, 30–3–?–p
Waddell, 60–10–45–a
Wager, 30–15–25–n
Walker (Walker's Variegated Free), 20–15–30–p
Ward's Late, 65–15–40–a
Washington Cling, 60–10–50–a
Waterloo, 20–0–40–p
Webb's Golden, 50–5–70–f
Wheatland, 40–10–?–a
Wheeler's Late, 75–5–25–a
Wilkins' Cling, 38–8–45–a
Willett, 50–15–30–a
Williams' Favorite, 40–10–23–p
Wilson's No. 14, 45–5–?–p
Wonderful, 35–15–0–n
Worth, 55–15–40–a
Wright, 5–3–30–n
Yellow Rareripe, 25–15–0–f

Plum

Abundance, 100–60–100–a–e
America, 40–20–20–p–s
American Eagle, 55–15–10–a–e
Apple, 90–40–?–a–m
Arkansas (Arkansas Lombard), 0–0–0–n–n
Assiniboia, 55–20–?–p–n
Best of All, 45–15–0–a–n
Broadshow, 0–0–?–n–n
Burbank, 100–60–100–a–e
Case Gold Drop, 0–0–?–n–n

Plum (*concluded*)

Chabot, 85–15–100–a–m
Clifford, 0–0–10–n–n
Cumberland, 20–0–0–a–m
Downing (Charles Downing), 55–20–0–n–m
Diamond, 0–0–?–n–n
Douglass, 80–20–0–a–m
Damson (Blue Damson), 0–0–?–n–n
Elapa, 55–20–0–n–n
Funk, 0–0–?–n–m
Gonzales, 95–30–?–a–m
Grand Duke, 0–0–?–n–n
Guthrie Topaz (Topaz), 63–25–?–n–n
Hale, 90–35–?–a–m
Hattankio (Hytankio), 50–15–10–a–m
Hawkeye, 50–15–0–p–n
Hungarian Prune, 0–0–?–p–n
Italian Prune, 0–0–0–n–n
Jefferson, 0–0–?–n–n
Jones Special, 75–25–30–n–n
Kerr, 60–25–?–a–m
Krap, 55–33–0–p–n
Kroh (Poole's Pride), 40–20–10–f–m
Lacy, 100–45–?–n–n
Lombard, 0–0–?–n–n
Maryland, 0–0–0–n–n
Milton, 0–0–0–n–n
Monarch, 50–20–?–p–n
Newman, 40–20–10–f–n
October (October Purple), 85–40–?–a–m
Ogon, 95–30–30–a–e
Orleans (Smith's Orleans), 0–0–?–n–n
Owanka, 55–15–?–n–n
Peter's Gage, 0–0–?–n–n
Pond (Hungarian Prune), 0–0–?–p–n
Pride (Shipper's Pride), 30–12–?–f–n
Purple Leaf, 90–35–?–a–m
Quaker, 45–15–50–p–m
Reagan, 55–25–0–n–n
Red Goose, 40–20–?–n–m
Red June, 95–25–100–a–e
Red Nagate, 100–50–?–a–m
Red October, 55–10–0–a–m
Reine Claude, 40–8–?–p–n
Robinson, 40–15–100–n–m
St. Martin (Coe's Late Red), 0–0–?–n–n
Shropshire (Shropshire Damson), 0–0–?–n–n
Sophie, 40–10–10–f–n
Stella, 95–55–?–p–n
Tokeya, 40–10–?–n–n
Wakapa, 55–15–?–n–n
Ward October, 40–10–20–p–m
Ward October Red, 45–15–0–a–m
Wastesa, 75–35–?–p–n
Whitaker, 0–0–0–n–m
Whiting, 0–0–?–n–m
Wildgoose, 40–10–0–n–m
Willard, 100–40–?–p–e
Winnipeg, 55–25–?–f–s
Wohanka, 50–25–?–n–n
Yellow Egg, 0–0–?–n–n
Yosemite, 90–30–10–a–e
Zekanta, 48–15–?–n–n

CONTROL

Observations and experiments in control were made principally with peaches and plums, as only a few varieties of apricots and nectarines were available for experimental work. This phase of the subject may be discussed under four heads: exclusion, eradication, protection, and immunization.

EXCLUSION

In budding, the greatest care should be exercised in selecting material only from trees known to be free from the organism. Even though the twigs are free from cankers, the organism may be transferred to the healthy stock through infected buds. Laboratory and field experiments made at the Missouri State Fruit Experiment Station show that it is impossible to determine from the external appearance of the bud whether it is infected.

In preparing to set out an orchard, great care should be used in ordering the trees. Purchases should be made only from reliable nurserymen, and, if possible, the nursery should be visited and the trees inspected in the fall, while they are still in full leaf, in order to make sure that the foliage is free from shot-hole condition and the twigs are free from cankers. If this is impossible, the trees should be carefully examined for black cankers in the spring, before they are set out. The disease is usually more easily detected in fall than in spring. All infected trees should be rejected.

Since the organism is readily carried from one tree to another, the location of a young orchard or nursery in relation to old, diseased trees or orchards is of considerable importance. The writer has frequently observed old orchards which served as centers of inoculum for young trees. In 1906 a tract of one hundred and sixty acres of new land near Koshkonong, Missouri, which bordered an old peach orchard on the south, was set with two-years-old Elberta peach trees. During the first year the foliage on the trees in the first fifteen rows became more or less diseased, but during the second year the disease advanced more rapidly and the foliage on trees in the two hundred and fifty rows next to the old orchard became involved. The trees beyond this, however, were normal and were making satisfactory growth. At the end of the third year nearly every tree in the entire orchard was diseased. Those in the first fifty rows bordering the old orchard contained more twig cankers than did those in any other part.

ERADICATION

After the disease has once become established, the chief point of attack is, of course, the cankers on twigs, limbs, and trunks of the trees, since these wounds not only serve to carry the organism through the winter but also in many cases tide it over unfavorable periods in summer. Careful pruning not only removes many of the twig cankers — and very often the cankers near the tips of the twigs contain the greatest numbers of bacteria — but also serves to stimulate new growth which often shows considerable resistance to the attacks of the organism. It is of course impossible to remove all the twig cankers in a crop season. But in an off year, when the fruit buds have all been winterkilled, the twig cankers on the old trees can be removed by cutting back the limbs to one-fourth of their entire length and carefully trimming out all the one- and two-years-old twigs from the center of the trees. Lesions on the limbs and trunks soon form rough, open cankers and can easily be found. Such wounds must also be cut out, or, if this is impossible, the diseased tissue must be completely removed by cutting around the canker well into the healthy part of the limb or the trunk. In every case the wound should be thoroughly disinfected with corrosive sublimate solution, one part to one thousand parts of water. The organism can usually be eliminated in this way on the apricot, the nectarine, and the peach. In the case of the plum, however, especially on the Japanese varieties where the organism has invaded the large limbs and the trunk, it is practically impossible to save the tree.

In 1907 the peach trees in the Missouri State Fruit Experiment Station variety orchard were heavily pruned. All the limbs were cut back one-fourth of their length. One hundred and thirty Elberta trees of the same age were not severely pruned but were treated in every other way as the other trees. During the fall the foliage on the pruned trees was of a better color and more nearly free from disease than that on the unpruned trees. In 1908 little or no injury was observed on the pruned trees, while foliage on all unpruned Elberta trees was more or less diseased. Twenty per cent of the leaves finally fell, and twig cankers also were plentiful. Chandler,[1] of the University of Missouri, has also observed in his work that severe pruning tends to make the trees more nearly free from the shot-hole disease.

PROTECTION

Spraying

Since bordeaux mixture has been used successfully in controlling various plant diseases, and lime-sulfur has been used also with more or less success, a number of experiments were undertaken in order to determine the effectiveness of these mixtures in controlling this organism.

In 1908, thirty-two eight-years-old Elberta peach trees were sprayed seven times with bordeaux mixture, and twelve were used as checks. The first application was made with the 6-4-50 formula on March 12, just before the fruit buds opened. The other six applications were made with the 2-9-50 formula after the leaves had unfolded; the first of these was made on April 21, and the others followed at intervals of from twelve to fifteen days. On August 12, the leaves, the twigs, and the fruit on the sprayed trees were free from disease. The fruit was of excellent color and quality, but there was sixty-one per cent less fruit on these trees than on the checks. The leaves on the checks were badly shot-holed and about ten per cent of them had fallen. The twigs also contained many blackened cankers. Sixty-three per cent of the fruit was infected.

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[1] Chandler, W. H., in a letter dated September 26, 1912.

In the same year, twenty-nine eight-years-old Elberta peach trees were sprayed seven times with lime-sulfur. Eleven trees were used as checks. The first application was made with the standard 15-15-50 formula on March 12, just before the buds opened. The other six applications were made with self-boiled lime-sulfur, 12-8-50 formula, after the leaves had opened; the first of these was made on April 21, and the others followed at intervals of from twelve to fifteen days. On August 12 the foliage on the sprayed trees was more or less dotted with diseased spots. The twigs also showed a few black cankers. Fifty-two per cent of the fruit contained many small bacterial wounds, but otherwise it was in good condition (Fig. 64). The yield was reduced twenty per cent. On the check trees the foliage was badly injured and ten per cent of the leaves had fallen. The twigs also showed many cankers. Sixty-three per cent of the fruit was diseased and cracked, its market value being considerably reduced.

In 1909, twenty-two nine-years-old Elberta peach trees were sprayed four times with peach bordeaux mixture, 2-9-50 formula. Twelve trees were used as checks. In 1908 curculio punctures made it difficult to determine the exact extent of injury due to the attack of this organism, and therefore in all the spraying experiments in 1909 arsenate of lead was added to the mixture used in the first two applications. The poison was added at the rate of two pounds to fifty gallons of the spray mixture. The first application was made about seven days after the petals had fallen, and the three others followed at intervals of four weeks. On August 8, as far as could be observed, the leaves, the twigs, and the fruit were free from disease. The foliage on the check trees was badly injured, and fifteen per cent of the leaves had fallen. The twigs contained many black cankers. Sixty-five per cent of the fruit was injured, and most of this was worthless.

In 1909, twenty-four nine-years-old Elberta peach trees were sprayed four times with self-boiled lime-sulfur, 12–8–50 formula. Arsenate of lead was added to the first two applications at the rate of two pounds of the poison to fifty gallons of the mixture. The first application was made about seven days after the petals had fallen, and the three others followed at intervals of four weeks. On August 8 only a few spots were observed on the leaves, and the twigs were apparently free from cankers, but fifteen per cent of the fruit was infected. The foliage on the check trees was badly injured, and fifteen per cent of the leaves had fallen. Many of the twigs were badly cankered. Sixty-three per cent of the fruit was infected, and most of this was worthless.

Apparently the arsenate of lead improved the effectiveness of the self-boiled lime-sulfur solution. It is quite probable, however, that the beneficial results obtained from the addition of arsenate of lead are due rather to the control of insects which serve as agents of dissemination than to the improvement of the mixture as a fungicide. Laboratory tests show that arsenate of lead used at the rate of two and one-half pounds to fifty gallons of water has very little influence on the growth of this organism.

From these and other experiments, it is evident that bordeaux mixture will control the disease. But the foliage of the trees, especially in the case of peach and nectarine, is extremely sensitive to the action of copper, and in some cases the trees are defoliated even when the copper is used at the rate of one-half pound of copper sulfate to nine pounds of lime and fifty gallons of water. Self-boiled lime-sulfur alone is much less effective, and in all the writer's experiments it failed to control the disease; but when two pounds of arsenate of lead were added to fifty gallons of the mixture it became much more effective.

Cultivation

A tract of about two hundred acres of new land at Koshkonong, Missouri, was set in 1906 with two-years-old Elberta peach trees which were more or less infected with the organism. Strips about four feet wide were plowed across the field and trees were set on the plowed land. The orchard was fairly well cultivated during the first three years. The organism gradually spread from the diseased to the healthy trees, and at the end of the third year every tree in the entire tract was more or less infected. Many of the trees were stunted and some of them finally died. Their death, however, could not be attributed directly to the work of this organism, but was apparently due to a combination of winter injury of the roots, premature defoliation due to the work of the bacteria, and, finally, the invasion of the roots by one of the root rot organisms. The trees of this orchard were uneven and produced very little fruit.

In the same year another tract of about one hundred acres of new land was planted with two-years-old Elberta trees brought from the same nursery. In this case the ground had been thoroughly prepared and the orchard was carefully cultivated during the following three years. A number of infected trees were observed in different parts of this orchard in the first year, but the disease spread very little and by the end of the third year all traces of disease had disappeared. The trees developed uniformly and produced large crops of excellent, clean fruit.

Diseased trees that have been properly cultivated invariably hold their leaves longer and resist the influence of the organism much better than do poorly cultivated trees. The general vigor of the host plays an important part in controlling the disease. Trees standing in thin, poor soil invariably suffer more severely than do those standing in more fertile places. Trees that yield abundantly are less resistant than those that produce little or no fruit. The foliage of infected trees that yield full crops in two consecutive years usually falls prematurely if the trees are not liberally fertilized and well cultivated.

In the spring of 1907 the peach trees in the Missouri State Fruit Experiment Station variety orchard were cut back severely, and about the middle of May complete fertilizer at the rate of 800 pounds to the acre was applied to the soil on one-half of the orchard. The same amount was applied also to an acre of Elberta peach trees of the same age which had not been cut back. Both the variety orchard and the Elberta orchard were finally planted in cowpeas. On September 1 the foliage on the trees that had been cut back and fertilized was in good condition and no premature defoliation was observed. The foliage on the Elberta trees, which had been fertilized and not cut back, was in fair condition, but from two to five per cent of the leaves had fallen. The foliage of the trees that had been cut back and not fertilized was more or less injured and from ten to twenty per cent of the leaves had fallen. The most striking results, however, appeared in September, 1908. The trees in the fertilized and cut-back block were practically free from disease. The foliage on the Elberta trees, which had been fertilized and not cut back, was more or less diseased, twenty per cent of the leaves had fallen, and many of the twigs contained black cankers. The foliage on the block of trees which had been cut back and not fertilized was much improved, not over two or three per cent of the leaves had fallen, and the twigs on very few of the trees contained cankers.

Chandler,[5] in a series of commercial fertilizer experiments conducted at Brandsville, Missouri, from 1910 to 1912, also has shown that the organism can be controlled to a large extent by the use of fertilizer. He obtained excellent results in using from 700 to 800 pounds of complete fertilizer per acre on mature trees and from 300 to 400 pounds on trees from two to three years old. Applications were made once a year in early spring or in June. Those made in June apparently gave better results than those made in spring. Chandler's results show that nitrate of soda is the essential element for controlling the disease. All plots that received nitrate of soda, whether alone or in combination with either potassium chloride or acid phosphate or both, showed very great resistance to the disease, but the plots receiving only potassium or phosphorus or both did not show any increased resistance. In the plots treated with nitrate of soda a few leaves were affected, yet very few fell as the result of the disease and the foliage had a healthy color. In some cases the trees of the check plot and those treated with potassium chloride and acid phosphate lost as high as fifty per cent of their leaves.

An application of manure well worked into the soil gives excellent results and in some cases the improved condition of the trees is more permanent than when commercial fertilizer alone is used. Good results have been obtained by seeding down the orchard permanently to red clover after a liberal application of manure. The clover is cut from time to time and allowed to rot under the trees. This practice, however, has disadvantages and cannot be recommended for general use. Very good results have been obtained also by following an application of manure with cowpeas. The tops are not cut but are allowed to stand until killed by frost, and the following spring the dead material is carefully worked into the soil. After the first year commercial fertilizers and cowpeas are used, but if the trees show marked improvement nitrate of soda is omitted from the fertilizer, since the cowpeas as a rule furnish sufficient nitrogen. This method gives good results. The peas, however, furnish an abundant food supply for mice, which are likely to injure the trees during the winter.

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[5] Chandler, W. H., in a letter to the writer dated September 26, 1912, containing a summary of some of his commercial fertilizer experiments with peach trees. A detailed account of the experiments will finally be published by the Missouri Experiment Station, Columbia, Missouri.

IMMUNIZATION

No doubt much can be accomplished in the way of resistance by crossing the less resistant with the more resistant varieties. This is especially true in the case of plums.

RECOMMENDATIONS FOR CONTROL

Since the organism is spread largely in the nursery, great care should be exercised in selecting trees for planting. Stock should be bought only from reliable nurserymen, who should be required to guarantee the trees to be free from the disease. A single diseased tree may cause serious loss after the trees are set out.

Old, neglected, budded apricot, nectarine, peach, and plum trees, especially plum, usually harbor the organism and become a menace to young orchard trees. All such trees should be cut down before the young trees are set out.

The ground must be thoroughly plowed before the young trees are set, and about one pound of a complete fertilizer should be worked into the soil about the tree while it is being set. Too much care cannot be given to the cultivation of an orchard, especially during the first two or three years.

Where the organism has become thoroughly established, systematic pruning, cutting out, and careful removing of all the diseased tissue from the cankers on the limbs and the trunks of the trees will eliminate the source of infection to a large extent. Complete eradication on old trees (seven or eight years old), however, can be accomplished only by pruning the trees. Pruning not only tends to eliminate the source of infection, but also serves to stimulate new growth which is more resistant to the disease.

Where the trees have been heavily pruned, a thorough application of bordeaux mixture (4 pounds of copper sulfate, 4 pounds of lump lime, and 50 gallons of water) just before the buds open will be of considerable assistance in controlling the disease on the fruit, especially if this application is followed by two or three applications of arsenate of lead ($2\frac{1}{2}$ pounds of arsenate of lead, 4 pounds of lump lime, and 50 gallons of water) to control the curculio and other insects, which aid materially in disseminating the organism. The first application of arsenate of lead should be made just as the shucks slip; the second, two weeks later; and the third, about two weeks after the second.

The older trees that are in good condition are as a rule not seriously injured by the disease. Thorough cultivation of an orchard and the use of fertilizers are absolutely essential. Complete fertilizer, at the rate of about 600 pounds to the acre in early spring for mature trees and from 150 to 300 pounds for young trees, gives very good results. In crop seasons a second application of about 250 to 300 pounds of the fertilizer should be made in June; the amount of nitrate of soda in this application should be reduced by about one-half. A liberal application of stable manure in the spring, well worked into the soil, will also give excellent results. Close attention to pruning and to the chemical and physical conditions of the soil will practically control this disease.

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THE DRY ROOT-ROT OF THE BEAN

WALTER H. BURKHOLDER

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THE DRY ROOT-ROT OF THE BEAN

THE DRY ROOT-ROT OF THE BEAN

WALTER H. BURKHOLDER[1]

Very little consideration has been given to the diseases that affect the bean plant (*Phaseolus vulgaris* L.) below ground. During the past few seasons an investigation of bean diseases in New York State by the writer has shown that such diseases are very common and frequently very destructive. The most important of these, and one that is enphytotic to the western bean section of the State, is the dry root-rot. This disease affects all the commercial varieties of the dry shell beans, and, so far as known, all those used for canning purposes. The dry root-rot may occur also on other species of Phaseolus. The following are affected: the tepary bean (*P. acutifolius* Gray var. *latifolius* Freeman), the scarlet runner bean (*P. multiflorus* Willd.), the moth bean (*P. aconitifolius* Jacq.), the lima bean (*P. lunatus* L.), and the adzuki bean (*P. angularis* Willd.). The disease has likewise been observed on the Black-eye cowpea (*Vigna sinensis* [L.] Endl.) and the kulti bean (*Dolichos biflorus* L.).

In New York State the disease is of general occurrence. It has been observed in 90 per cent of the bean fields of the six largest bean-producing counties, and is found wherever the crop has been grown to any extent. Specimens of bean plants affected with the dry root-rot have been received from A. H. Gilbert and G. A. Meckstroth, of the United States Bureau of Plant Industry, collected at Burlington, Vermont, and Grand Rapids, Michigan, respectively.

It is not known when the dry root-rot first appeared in New York State. A few growers claim to have observed it for at least twenty-five years. Since it is now of such general distribution in the bean sections, apparently it must have been present for a long time. As far as known, no reference to this disease has ever been made in literature except by the writer (1916 and 1917), and these references apply to New York State. The disease therefore evidently originated in this region or was introduced from a locality where it has not attracted attention. The symptoms of the disease above ground are not striking and could readily be mistaken for the result of unfavorable weather conditions. Thus, where the disease is not abundant it could easily be overlooked.

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[1] The writer wishes to acknowledge his indebtedness to Dr. Donald Reddick for helpful suggestions and criticisms during the progress of this work.

In New York State where the dry root-rot is common, severe losses to the bean crop result. It is a matter of common observation that there has been during the past ten years a marked decrease in the bean yield. The decrease has been estimated by a number of the large growers and seedsmen to be approximately 25 per cent. The writer, from observations in the bean section, is led to the conclusion that no small part of this decrease is due to the dry root-rot.

SYMPTOMS OF THE DISEASE

ON THE ROOTS AND OTHER UNDERGROUND PARTS

The first signs of the disease on the bean show a week or two after the plant has appeared above ground. At this time there may be observed on the taproot a reddish discoloration, but the morphology of the root remains normal. The discoloration may cover the taproot and have no

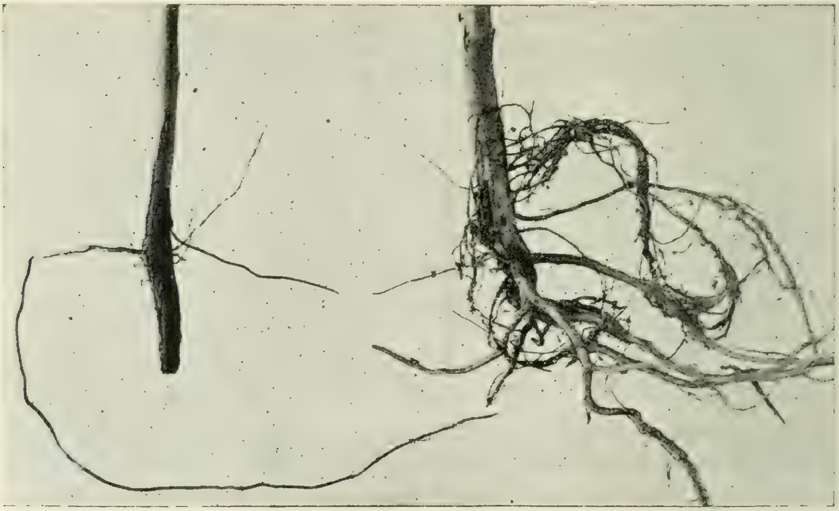
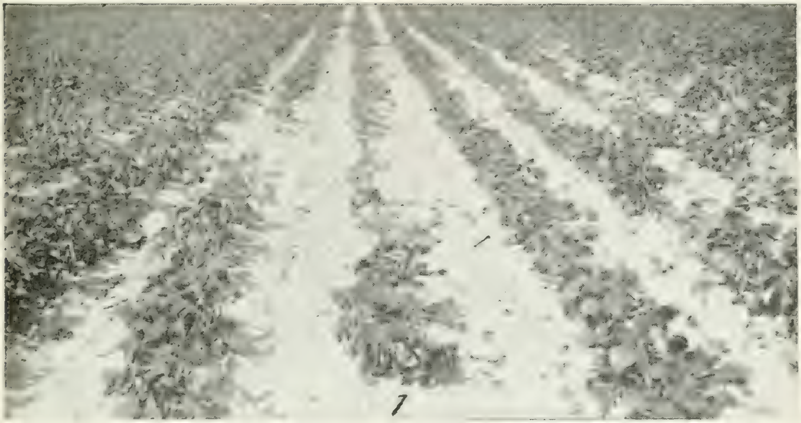
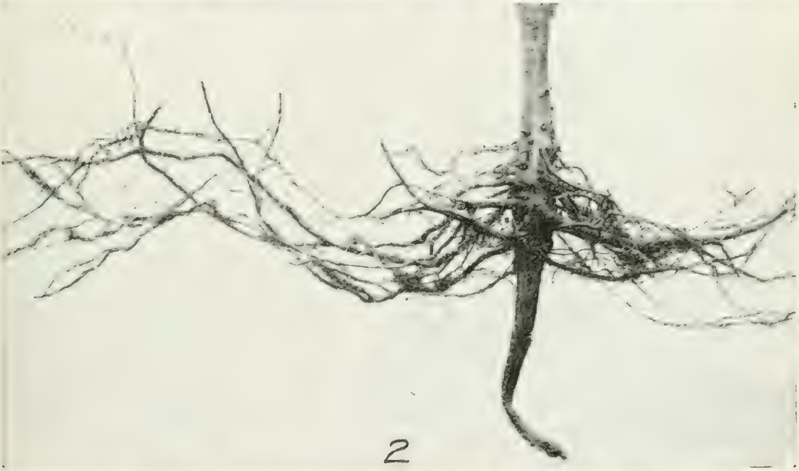
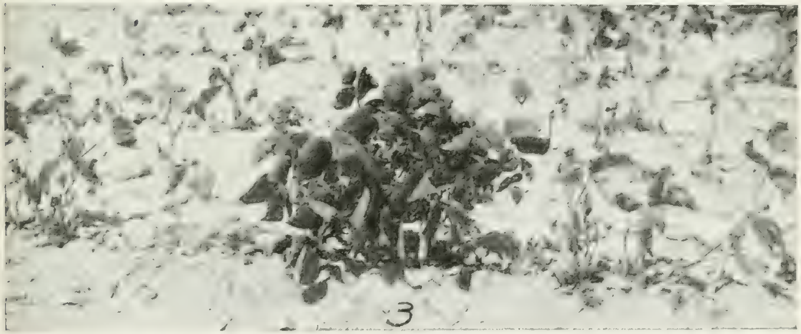


FIG. 133. THE DRY ROOT-ROT OF THE BEAN

At left, root of a White Marrow bean affected with dry root-rot. At right, a healthy root; the strong lateral roots branching off from the lower end of the taproot are evident





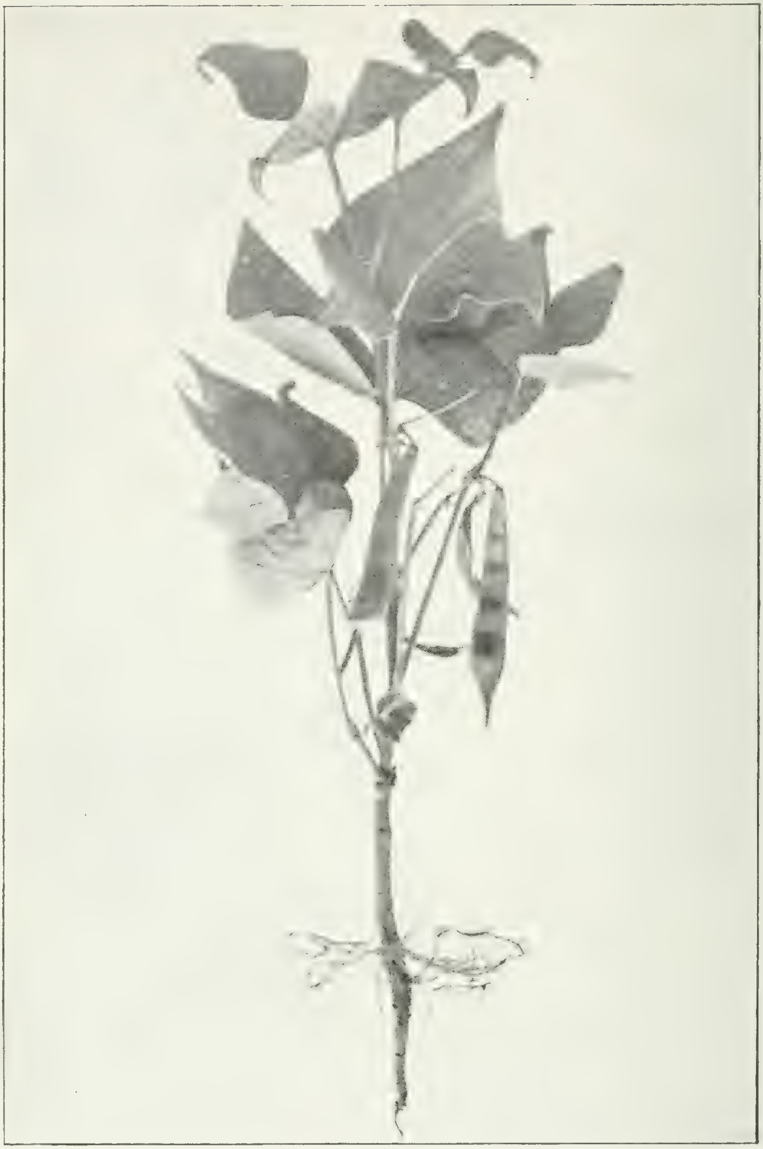


DRY ROOT-ROT OF BEAN

1, A field of White Marrow beans affected with dry root-rot. The rows are 28 inches apart. Healthy plants would cover the ground

2, The dry root-rot of the bean. A mass of surface roots has been produced above the dried taproot

3, Flat Marrow bean resistant to dry root-rot. The effect of the disease is shown on the White Marrows near the Flat Marrow



EFFECT OF DRY ROOT-ROT ON THE PARTS OF THE WHITE MARROW BEAN PLANT ABOVE GROUND

The diseased plant has failed to vine. Also the pods are few in number and are poorly filled definite margin, or may occur as red streaks which frequently extend to or above the surface of the ground. Later the diseased areas become brown and longitudinal fissures appear in the cortex.

As the disease progresses upward, the lower lateral roots and the end of the taproot shrivel and become dry. Frequently, too, the main root and the lower part of the stalk are found to be pithy. Above the dead area new lateral roots are developed. These frequently push their way through the diseased cortex and subsequently become diseased. Other roots that are produced above the lesions may develop rapidly and become abnormally large, to take the place of the diseased lower rootlets. Occasionally one of these lateral roots takes the place of the taproot. More frequently, however, these surface roots are small and very numerous, and form a dense mat in the first inch or so of soil (Plate LVI, 2). As a rule they do not become severely affected, and persist throughout the entire growing season. On the other hand, in severe cases the entire root system may be destroyed.

ON THE PLANT ABOVE GROUND

Although no lesions appear above ground, the effect of the loss of the lower lateral roots and part of the taproot is very noticeable. This is especially true during the latter part of the growing season. During the first five to six weeks of growth a diseased plant may not be readily detected unless in comparison with a healthy one. At this stage the affected plant is slightly dwarfed but otherwise remains normal in appearance so far as the parts above ground are concerned. The symptoms of the disease are more evident at podding time. There is then an apparent checking in growth of the entire plant. Few pods are formed, and the remainder either fail to set, or drop in the early stages of their formation (Plate LVII). The leaves of plants affected with the dry root-rot frequently turn yellow and fall. This has given rise in some localities to the name *yellow-leaf*, but it is a character which is not constant. Nevertheless, the diseased individuals mature earlier than do normal plants, since they dry much more rapidly in the absence of an entire root system. The seeds also in the few pods frequently are under size, and this contributes to the loss in yield.

In the true sense of the word this disease is not a wilt, as the leaves or the tender parts of the plant seldom flag. In a few cases, however, the writer has observed a distinct wilting of the plants. In these latter cases all the leaves dropped and turned brown, and persisted on the plant. The small pods withered and clung to the stem, while the older pods matured a few small seeds. A condition similar to this has frequently been observed which is due to the bacterial blight caused by *Bacterium phaseoli* E. F. Smith. Careful examination, however, may distinguish between the two diseases.

The general appearance of fields of beans affected with the dry root-rot may vary. Whenever the disease occurs in a field, approximately 100 per cent of the plants show the symptoms. All the plants may appear uniform (Plate LVI, 1); on the other hand, certain parts of the field frequently are affected more severely than others, thus giving the appearance that the disease occurs in limited areas. There are several possible explanations for this spotted condition. One is that the soil conditions are not uniform; a second is that frequently wireworms or other insects attack the decaying roots and complete their destruction.

SYMPTOMS OF OTHER ROOT DISEASES OF THE BEAN

Two other root diseases of the bean in New York State might be confused with the dry root-rot. They are the black root-rot, caused by *Thielavia basicola* Zopf., and a blotch caused by Rhizoctonia. In the greenhouse, species of Botrytis, Fusarium, and various other fungi attack the young seedling at the surface of the ground or cause more or less of a root disturbance. These fungi have seldom been observed in the field.

Black root-rot

The black root-rot in New York State is local in its distribution. It has been observed only along the southern edge of the dry-shell-bean district. In its early stages this disease is scarcely distinguishable from the dry root-rot. The discoloration of the taproot at times may appear to be somewhat purple in contrast to the orange-red color produced by the dry root-rot. Otherwise the two diseases are very similar. Later in the season, coal-black lesions, which are very characteristic, appear on the affected roots. These lesions may vary from small streaks to cankers which encircle half the taproot and completely envelop many of the lateral roots. Frequently during damp weather a frosty appearance may be noticed over the lesions and the diseased parts of the plants. This is due to the large production of the so-called endoconidiophores belonging to *Thielavia basicola.* Since this disease is often observed associated with the dry root-rot, the two groups of symptoms may be found together.

It has been the observation of the writer that the black root-rot occurs more generally during the early part of the growing season, and at times affects germination. Frequently plants affected with this disease may be able to throw it off by the middle of August, and late infection causes no serious injury to the root system. These two observations have been further substantiated by the writer through inoculation experiments. The symptom of the dry root-rot, on the other hand, is more evident during the latter part of the season.

Rhizoctonia blotch

The Rhizoctonia blotch in New York State is of general distribution but has never been observed to cause any appreciable losses. Brick-red blotches are produced mainly on the taproot and on the bean stem near the surface of the ground. The lateral roots are seldom affected. The lesions caused by Rhizoctonia are sunken, and usually irregular but definite in outline. This is the most distinguishing characteristic of the disease, and as a rule readily separates it from the other root diseases of the bean.

ETIOLOGY

The dry root-rot disease of the bean is caused by the fungous pathogene *Fusarium martii phaseoli* n. form.

MORPHOLOGY

The pathogene causing dry root-rot of the bean has the following morphological characters:

Macroconidia mostly 3-septate (44.5 x 5.1 μ), 4-septate (50.09 x 5.3 μ), rarely 5-septate, of nearly even diameter throughout, more or less curved near apex, with somewhat rounded or but slightly pointed apex, usually apedicellate. Microconidia rare. Aerial mycelium in culture scanty and usually white. Spores borne mostly in pseudopionnotes. Cultures when mature from a lichen[2] and montpellier green (on synthetic agar [3]) to a pale olive-buff or cinnabar-green (on potato agar), to a zinc-green and dusky green-blue (on potato plug), to a purple-drab (on steamed rice). Spores in mass frequently yellowish. Chlamydospores terminal or intercalary, single or in short chains (11.6 μ in diameter).

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[2] The colors are after Robert Ridgway's *Color Standards and Color Nomenclature.*

[3] Richard's solution with two per cent agar.

CULTURAL CHARACTERS

In growing the fungus on various media, a marked variation in its microscopical appearance is seldom found. The intensity of color production, however, varies considerably, not only on different media but on the same medium with the presence or the absence of air. The colors, when produced, are of various shades of blue and green. The spore mass frequently has a yellowish appearance. The growth of *Fusarium martii phaseoli* on steamed rice differs greatly from that on any other medium used. The fungus on rice is purple-drab but the medium itself is colored a coral pink. Microscopically also there is a great change. The spores are very abnormal and a great number of microconidia are produced. On cellulose agar the fungous growth is very scanty and there is scarcely any digestion of the cellulose.

The proportion of conidium types (those bearing the same septations) and the conidium measurements do not vary greatly on the different agars and on sterilized vegetables. For making the measurements, the fungus was grown on six different media. In the majority of cases the media were the same as those used by Sherbakoff (1915 b) in his study of *Fusarium martii* Ap. and Wr. The age of the cultures also was similar; in most cases they were in a state of maturity. One hundred conidia taken at random were measured in each case. Types that occurred in a culture but were not found when measuring the spores were marked "rare." It was considered that such types were present in less than one per cent.

The measurements of the conidia were as follows:

On bean plugs, cultures eighty-three days old; conidia taken from pseudopionnotes:

Conidia: 3–septate, 38 per cent, 41.4 x 4.9 (37.7–46.5 x 3.9–5.8) μ
4–septate, 62 per cent, 47 x 5 (40.3–53.3 x 4.5–5.8) μ
5–septate, rare

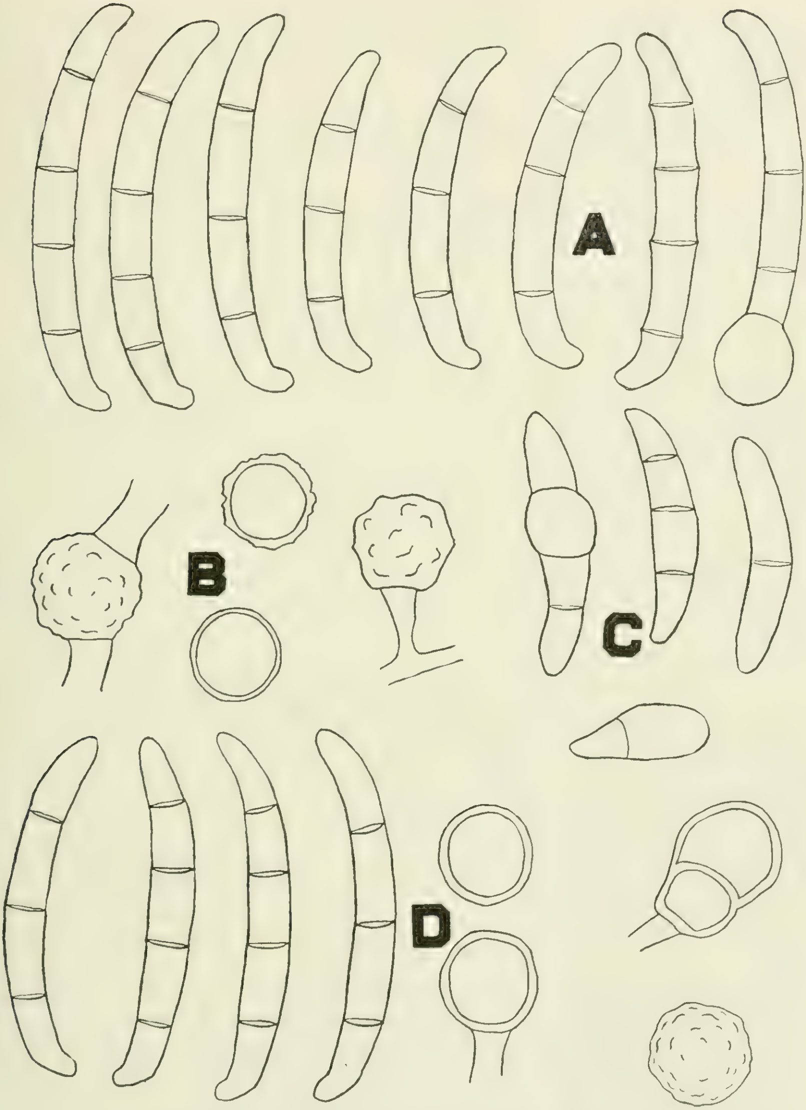

FIG. 134. SPORES OF FUSARIUM MARTII PHASEOLI

A, Macroconidia from sterilized bean plug cultures 83 days old; B, chlamydospores from the same culture; C, macroconidia from steamed rice, cultures 80 days old; D, macroconidia and chlamydospores from potato agar cultures 70 days old. Camera lucida drawings, × 1027

On raspberry cane plugs, cultures eighty days old; conidia taken from pseudopionnotes:

Conidia: 2–septate, rare
3–septate, 66 per cent, 41.4 x 5 (32–48 x 4–5.8) μ
4–septate, 34 per cent, 49.3 x 5 (44–56 x 4–5.8) μ

On potato agar containing one per cent glucose, cultures twenty-tw days old; conidia from pseudopionnotes:

Conidia: 2–septate, 3 per cent, 37.7 x 4.4 (36–40 x 4–5.3) μ
3-septate, 83 per cent, 43.5 x 5.2 (33.3–56 x 4–6.6) μ
4–septate, 14 per cent, 52.3 x 5.4 (42.6–60 x 4.6–6.6) μ

On slightly acidified hard potato agar, cultures eleven days old; conidia from pseudopionnotes:

Conidia: 3–septate, 24 per cent, 48.7 x 5.3 (35.3–53.3 x 4.6–6.6) μ
4–septate, 76 per cent, 52.9 x 5.5 (46.6–56 x 4.6–8) μ
5–septate, rare

On potato plug, cultures eighty-three days old; conidia from pseudopionnotes:

Conidia: 2–septate, rare
3–septate, 76 per cent, 43.5 x 5.2 (36–48 x 4–8) μ
4–septate, 24 per cent, 46 x 5.5 (42–49.3 x 4.6–6.6) μ

On synthetic agar,[4] cultures thirty-three days old; conidia from pseudopionnotes:

Conidia: 3–septate, 24 per cent, 50.7 x 5.1 (41.6–58.5 x 4.5–6.5) μ
4–septate, 76 per cent, 54 x 5.2 (29.9–59.8 x 3.9–6.5) μ
5–septate, rare

NOMENCLATURE

The name *Fusarium martii* was first used by Appel and Wollenweber (1913) to describe a fungus which they regarded as identical with *Fusisporium solani* Martius. These investigators presented a detailed description of the species, as did also Sherbakoff (1915 b) a few years later in his monograph on the Fusaria of potatoes. In both instances the fungus was found on decaying potato tubers and was considered to be saprophytic on that host. On the other hand, Carpenter (1915) refers to a culture of *Fusarium martii* isolated from *Pisum sativum* by Westerdijk and determined by Wollenweber, which evidently was considered to be parasitic since it was thought by Miss Westerdijk to be *F. vasinfectum* var. *pisi* Van Hall, the cause of the St. John's sickness of garden peas. So far as is known, no inoculation experiments were made. Wollenweber shows drawings of this strain in *Fusaria autographia delineata*, issued in 1916.

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[4] Richard's solution with two per cent agar.

The fungus isolated from diseased bean roots, and proved to be parasitic on this host, is practically identical with *F. martii.* The spore measurements are approximately the same. The color production is identical with that of *F. martii* and the pathogene in appearance agrees remarkably well with the plates given by Carpenter (1915). The number of septa in the conidia, and the proportion of conidia having the same number, also are similar. One slight difference was observed. Appel and Wollenweber state that 5-septate spores are rare, but they find a sufficient number to give spore measurements and drawings. Sherbakoff likewise finds as many as seven per cent of 5-septate spores on some media. In the species under consideration, 5-septate spores were found in less than one per cent. The fact that they occur in fewer numbers, however, seems of small consequence and can scarcely be pointed to as a distinct difference in the two organisms. Moreover, a culture of the fungus from affected bean roots was sent for determination to Dr. Sherbakoff, who states, in a letter to the writer, that this fungus "is morphologically the same as *F. martii* because of the same macroscopical characters, the same type and size of conidia and of the same chlamydospores." Slight differences that might occur he considers not sufficient to separate this fungus from the above species.

For comparison of the fungus under consideration with *F. martii,* cultures of the latter were obtained at various times from several different sources. The first culture received was from R. J. Haskell and was originally from Dr. Sherbakoff, the latter having discarded his own cultures; a second culture was supplied by the Bureau of Plant Industry from Wollenweber's collection; a third was from J. Westerdijk at the Centralstelle für Pilzkulturen. The macroscopical appearance of subcultures from these were slightly different from the organism from bean root. There was also a slight difference in appearance among the three strains of *F. martii.* The difference lay in intensity of color, greater or less amount of aerial mycelium, and variations in the formation of pseudopionnotes. Spore measurements made from the culture obtained from the Wollenweber collection varied considerably from the measurements given by Appel and Wollenweber and by Sherbakoff. The cultures from the Centralstelle für Pilzkulturen, however, agreed very closely with the original measurements. Unfortunately the strain isolated from potato by Dr. Sherbakoff was lost before spore measurements could be made. Inoculation experiments, however, were conducted with all three strains with the bean as the host plant. In no case did infection occur. It is very improbable that these negative results are due to unfavorable conditions, as positive results were always obtained at the same time with the fungus from bean roots. There is no evidence that *F. martii* is parasitic on the bean root.

From the foregoing statements it seems scarcely possible that the bean Fusarium is identical with *F. martii.* There is a distinct physiological difference, and possibly slight morphological differences. The latter are so unimportant that the writer does not feel justified in using them as the basis of a new species or even a new variety. Physiological differences, however, have been used in this genus as the basis of new varieties. *Fusarium vasinfectum* var. *inodoratum* Wr. differs from *F. vasinfectum* Atk. only by the absence of odor. On the other hand, in other groups it has been the custom, when the physiological difference was one of pathogenicity, to treat the new form as a biologic species. For this reason the writer has used the trinomial *Fusarium martii phaseoli.*

Since one strain of *F. martii* was isolated from a diseased pea root by Dr. Westerdijk, there is the probability of another biologic species, *F. martii pisi.* The writer, however, obtained no infections on the garden pea when inoculations were made with *F. martii.* The original species appears to be saprophytic.

LIFE HISTORY

The time of bean planting in New York State varies according to weather conditions, but usually the seed is put into the ground in the first half of June. The soil at that time is in a warm and moist condition. Thus as soon as germination of the bean seed takes place, external factors are favorable for the infection of the young roots by the dry root-rot fungus. The taproot, being the first to develop, soon shows symptoms of the disease. This is frequently a week or two after the host plant is above ground. It has been determined by experiment that further infections may take place at any time during the growing season of the bean plant.

The exact mode of infection is not known. The fungus, however, may penetrate the healthy epidermis. After the mycelium has once gained entrance to the host, it is for a short time intercellular in the cortical tissue. In the main the hyphae are found extending longitudinally with the taproot. Occasionally as many as a dozen hyphae may be observed growing parallel in the intercellular spaces (fig. 135). This tendency on the part of the fungus to form rhizomorph-like structures is further developed on the outside of the host, where strands 75 μ in diameter frequently occur over the diseased areas. Likewise, in culture,

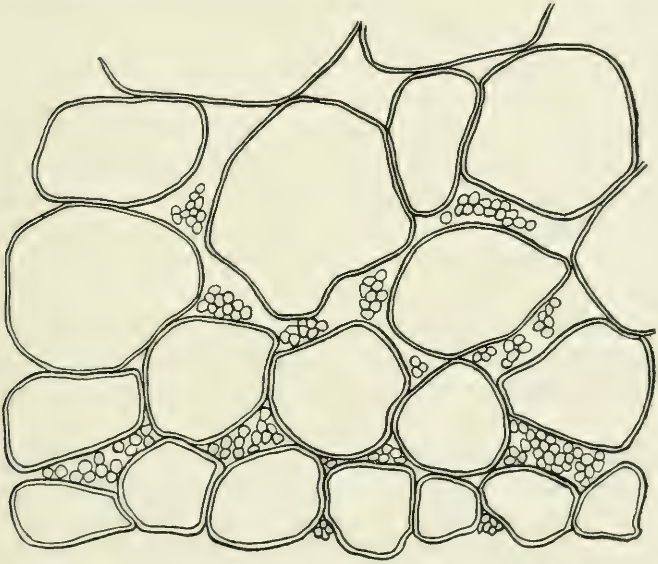

FIG. 135. CROSS SECTION OF THE CORTEX AND EPIDERMIS OF THE TAPROOT OF A BEAN AFFECTED WITH DRY ROOT-ROT

An early stage in the progress of the disease, showing the intercellular and parallel hyphae. Camera lucida drawing, × 514

Fusarium martii phaseoli produces these strands, which at times develop into coremium-like structures of fructification.

The mycelium in the host rarely extends any distance above the surface of the soil. In its growth in the cortex it possibly reaches its greatest height; here, at times, the coloration due to the fungus may appear on the lower part of the stem. The mycelium in the cortex of the upper part of the root does not penetrate into the vascular bundles, hyphae having been observed to enter the vascular system only through the small lateral rootlets on the lower part of the taproot. The mycelium does not long remain intercellular, but soon enters the cells of all the tissue. From the lower part of the taproot the hyphae progress upward through the cells of the vascular system. Frequently they fill these cells or cause them to collapse, but seldom is a wilting of the host plant produced. The growth of the fungus is extremely slow; by harvest time of the host it frequently has not reached within an inch of the surface of the soil. The height that it attains in the vascular tissue, however, depends on the time of infection.

F. martii phaseoli produces two kinds of spores on and in the bean roots. They are the chlamydospores and the conidia of the fungus. The chlamydospores are borne singly, or, less frequently, in small chains within the host tissue. For the most part they are found in the cortex. In the smaller fiber-like rootlets they occur in greater numbers than in the larger lateral roots and in the taproot. This is due probably to an absence of sufficient food material in the smaller roots. Repeated attempts to germinate these spores have failed. Evidently the optimum set of conditions governing germination has not been discovered. Conidia are rarely produced in abundance on the host during the growing season. Frequently spores cannot be found even on severely diseased roots. Before the cause of the dry root-rot was determined, many specimens of infected bean roots were examined without success in finding the spores of the causal organism. A few instances only have been observed of large production of conidia on the dead parts of affected roots. Such cases were during rainy weather, and then the production was so great that the roots appeared whitish. Apparently a sufficient number are always produced for the dissemination of the fungus. In many of the infested fields a small quantity of soil will contain the pathogene to such an extent as to cause immediate infection of bean seedlings. This, however, might be due to the mycelium.

From observations it appears that as a rule the fungus is disseminated in the manure used to fertilize the bean fields. It is the practice of growers to pull the bean plants in the late summer, and, after threshing the seed, to use the remainder of the plant as bean straw. This is fed to cattle and sheep and is one of the valuable by-products of the bean industry. The straw contains the diseased bean roots, which are seldom eaten but are thrown into the compost heap where they undergo further decomposition. The fungus here lives saprophytically and produces its spores on old bean roots and stems. When the manure is spread over the fields the pathogene also is carried to new areas. A striking instance of this was observed on a farm near Perry, New York. During the latter part of the growing season of 1916, the location could be determined where four loads of manure had been distributed in the bean field. This was due to the fact that the dry root-rot was more severe in these places.

In the sections where snap beans are grown, this method of dissemination of the fungus does not apply. Here, after the crop is harvested, the vines are plowed under, not pulled for straw. Thus the fungus does not get into the manure, and therefore is not distributed so readily over the fields.

The pathogene may be carried from one field to another in various other ways. It may be disseminated on the feet of horses and of men, and on tillage instruments. It may be spread by the wind, blowing dust from field to field. Observations in the greenhouse indicate that it can be carried by insects. Another means of dissemination is by washing. In the valleys about Warsaw and Attica, New York, all the fields are equally contaminated with *F. martii phaseoli*, whether or not they have been planted to beans or have had bean-straw manure applied to them. It is known, however, that in certain years these fields have been under water during the early spring. At such periods it is very probable that the pathogene is carried from field to field along the valleys.

There are no indications that the fungus is carried with the seed, although if the seed is not well cleaned it may harbor small pieces of the diseased roots and particles of dirt containing the pathogene.

The dry root-rot fungus may overwinter in the bean straw or in the compost heap, and in the following spring it is returned to the fields.

This is a common method of hibernation, but it does not account for the presence of the fungus in the majority of fields in the bean section of New York State. The pathogene when it once gains entrance into a field is very persistent, and may live for a number of years as a saprophyte in the soil. Since the common practice in the bean section is a three-years rotation, the fungus is known to exist for such a period in the absence of the host plant. Observations indicate further that it may live for as long a period as ten years. In the laboratory, in soil to which no moisture was added, the pathogene died within a period of two years. In addition to water, decaying organic matter is no doubt necessary for the persistence of the pathogene in a saprophytic condition.

Only one experiment has been conducted which has any bearing on the length of time the pathogene may live in the soil under natural conditions. This experiment was begun in 1915 and data were taken at the end of three years. Four rows of beans, approximately 100 feet long, were inoculated with the fungus *F. martii phaseoli*. Four rows were planted as a check to determine whether the pathogene was already inhabiting the soil. The plants of the check rows remained healthy. At the end of the season the bean plants were cut off and the roots were allowed to remain in the soil. The plot was then dragged over and sown to wheat and clover, both red clover and alsike (*Trifolium pratense* and *T. hybridum*) being used in the seeding. After the wheat was cut in 1916, the clover remained on the plot until the spring of 1918, when the plot was planted to potatoes. In October of 1918 soil was taken from the plot where the diseased plants had been, and was placed in sixteen pots in the greenhouse. Three seeds of the Red Kidney variety of beans were placed in each pot. At the end of five weeks the roots of the plants were examined. In no case were lesions of the dry root-rot observed on them. The results of this experiment are contrary to all observations of the writer in the field. Moreover, the test is small and inconclusive, and is given here merely for what it is worth.

In just what form the pathogene persists during the winter and in the absence of its host plant has not been determined. It is probably in the mycelial stage during favorable conditions and in the chlamydospore stage during adverse conditions. Repeated examination of overwintered matter has never revealed the presence of a sexual stage.

PATHOGENICITY

Inoculation experiments with the bean plant

Many hundreds of inoculations have been made from time to time with *Fusarium martii phaseoli* on the roots of bean plants. In approximately one hundred per cent of the tests, infections occurred. These experiments were conducted both in the greenhouse and in experimental gardens. Infection takes place readily when the soil is contaminated before the seed is planted, when the seeds are dipped in suspensions of spores from a pure culture and planted in sterilized soil, and when inoculations are made with the pathogene after the plants are above ground. The incubation period under favorable conditions is about five days to a week. The spores used in these experiments were taken from pure cultures and always showed a high percentage of germination. The age of the cultures varied greatly; this factor, however, is unimportant, since the spores do not lose their viability very early.

Inoculation experiments were conducted to determine other important points respecting the pathogenicity of the fungus. The length of the period of susceptibility in the bean plant was thought to be worthy of consideration. It was believed that if it were found that infection takes place only in the seedling stage, a basis for controlling the disease would be available. It might be possible to protect the bean roots from the pathogene for a short time. On the other hand, the extermination of the fungus in the soil is exceedingly difficult without injury to the host plant or without too great expense. It was found, however, that the bean plants were susceptible over a greater period than the seedling stage. The results of the experiment to determine this point are set forth in table 1. The seeds used in the experiment were of the White Marrow variety.

The plants inoculated thirty-one days after planting became infected as readily as did those inoculated at the time of planting. From this it is evident that the period of infection does not extend over the seedling stage only, but on toward the maturity of the plant. It is possible that the plants are susceptible throughout their period of life. Early infection, however, would be more severe, as the fungus grows but slowly in the host.

TABLE 1. RESULTS OF AN EXPERIMENT TO DETERMINE THE PERIOD OF SUSCEPTIBILITY OF THE BEAN PLANT TO FUSARIUM MARTII PHASEOLI

Date of planting (1915)	Number of days until inoculation	Number of plants	Number of infections
July 14	None	33	33
July 30	Check	35	0
July 30	8 days	40	40
July 30	Check	40	0
July 30	16 days	40	39
July 30	Check	45	0
July 31	31 days	36	36
July 31	Check	36	0

An experiment was conducted to determine whether the pathogene can live over winter in the compost heap and be carried back to the land. For this experiment manure was procured from a sheep barn where bean straw had been fed. The experiment was run in duplicate, one plot being at Perry and one at Ithaca. It is frequently almost impossible in certain counties to obtain land free from this pathogene. Since Ithaca is out of the bean-growing district, it was more or less certain that the land there would not be contaminated with the fungus. The experimental plot at Perry was situated in the center of an old apple orchard and also proved to be free from the organism.

In each plot two rows of beans, of approximately 150 plants each, were used. In some instances the seed-corn maggot (*Phorbia fusciceps* Zett.) and the slug (*Agriolimax* sp.) destroyed a number of plants. One row

TABLE 2. RESULTS OF AN EXPERIMENT TO DETERMINE WHETHER FUSARIUM MARTII PHASEOLI MAY WINTER IN THE COMPOST HEAP AND BE CARRIED BACK TO THE LAND THE FOLLOWING SEASON

	Number of healthy plants	Number of diseased plants
Plots at Ithaca		
Treated row	47	40
Check row	147	2
Plots at Perry		
Treated row	38	107
Check row	96	3

was used as a check row, while the other was heavily fertilized with the bean-straw manure. The manure contained decaying pieces of bean stems and roots which had been in the compost heap during the preceding winter. The plots at Ithaca were planted on June 22, 1916, and those at Perry were planted on June 28, 1916. The results of the experiment are shown in table 2.

The few diseased plants appearing in the check rows can be accounted for readily as infections caused by washings from the diseased rows. The fungus also could have been transferred during cultivation.

Cross-inoculations

Many inoculation experiments were conducted to find other hosts of *Fusarium martii phaseoli.* These experiments were conducted both in the greenhouse and in the garden. In all cases rows of beans were inoculated with the pathogene as a check. Without exception infection was obtained on the bean. The following plants proved susceptible to *F. martii phaseoli*: the tepary bean (*Phaseolus acutifolius* Gray var. *latifolius* Freeman), the scarlet runner bean (*P. multiflorus* Willd.), the adzuki bean (*P. angularis* Willd.), the moth bean (*P. aconitifolius* Jacq.), the Black-eye cowpea (*Vigna sinensis* [L.] Endl.), and the kulti bean (*Dolichos biflorus* L.). Infection was very light on the scarlet runner and the lima bean.

Negative results were obtained with a number of legumes and other plants. This list is as follows: the garden pea (*Pisum sativum* L.); the field pea (*P. sativum* L. var. *arvense* Poir.); red clover (*Trifolium pratense* L.); alsike clover (*T. hybridum* L.); vetch (*Vicia* sp.); the following varieties of soybeans (*Soja max* Piper)— Medium Green, Ito San, Auburn, Wilson; corn (*Zea mays* L.); potato (*Solanum tuberosum* L.); oats (*Avena sativa* L.); wheat (*Triticum* sp.); and the following weeds —*Ambrosia artemisiifolia* L., *Prunella vulgaris* L., *Chenopodium album* L., *Rumex* sp. The weeds used in the inoculation experiments were those found commonly in the bean fields.

EFFECT OF WEATHER CONDITIONS

In the case of root parasites, infection is not so dependent on weather conditions as with those pathogenes which attack parts of plants above ground. Such factors as sun, wind, rain, and dews are eliminated.

Except under very prolonged drought there is sufficient moisture in the soil for spores to germinate and infect the host. Temperature appears to be the chief limiting factor, and from recent articles on the subject this varies greatly with the pathogene and the host. Gilman (1914 and 1916) considers high temperature favorable to infection of cabbage by *Fusarium conglutinans* Wr. Tisdale (1917 a) came to similar conclusions in his observations on the infection of flax by *Fusarium lini* Bolley, and states that from 15° to 16° C. is the minimum temperature at which infection will occur. Ramsey (1918), on the other hand, is of the opinion that a cool, moist soil is essential for infection by *Spongospora subterranea* (Wallr.) Johnson to occur on potato tubers.

In working with *Fusarium martii phaseoli*, the organism under consideration, Reddick (1917) found that infection occurred between temperatures of 15° and 34° C. His results have been substantiated by the writer. These temperatures include, with the exception of a few cases, the limits of the soil temperature found to exist at Perry, New York, during the growing seasons of 1916 and 1917. Records were taken at that place with a continuous self-recording soil thermograph. In no case was the temperature recorded higher than 30° C., and only a few times did it drop below 15° C.; in the latter instances it always rose immediately. From these data it is apparent that the soil temperature about Perry, which is near the center of the bean district, is always sufficient for the bean plant to become infected with *F. martii phaseoli.*

Besides the effect that soil temperature has on infection, there is also its effect on the progress of the disease after infection has once taken place. The latter is possibly of the greater importance. The problem here is more complex and very few definite conclusions have been drawn pertaining to it. Reddick, in the article cited above, has shown that soil temperature influences very greatly the growth of the bean plant. High temperatures allow much more rapid yet healthy growth than do low temperatures. Thus it is difficult to analyze data concerning the effect of the disease at different temperatures of the soil, since the rate of growth of the host plant varies so much. There were, however, in experiments conducted under these conditions, indications that the disease is more severe at a soil temperature of 22° C. than at 34° C. The former temperature is nearer the average of the soil temperature found in the bean district during the growing seasons of 1916 and 1917.

Even more marked than the effect of soil temperature on the disease is the effect of varying amounts of soil moisture. As already stated, the pathogene *F. martii phaseoli* is not a wilt-producing organism although the mycelium frequently invades the vascular system. The effect of the fungus is rather to kill and dry up the tissue as it progresses up the root, thus greatly reducing the root system of the plant. The roots that remain healthy or are able to carry on their normal functions are the surface roots. If there is an abundance of water in the soil, these surface roots can supply the plant with sufficient moisture. As the percentage of soil moisture decreases, however, this ability of the surface roots to supply moisture also decreases. A drought of ten days or two weeks will bring forth from the growers the statement that their beans are " going back." The expression is descriptive. The symptoms of the disease become very evident in a relatively short time. If a prolonged dry spell occurs at the time of blossoming and pod production, it is very injurious to the diseased plants. The yield, without doubt, will be reduced over fifty per cent. On the other hand, a very high moisture content of the soil aids materially in the dissemination of the fungus. Moisture also causes a more rapid progress of the disease in the host, since the diseased parts, which are usually dry, absorb water very readily. In this way sufficient moisture is supplied to the pathogene. A continuous rainy season, however, does not cause a noticeable reduction in yield of diseased plants. During the season of 1915 a field of White Marrow beans near Wyoming, New York, was under the observation of the writer. The plants on this field were severely infected, and at podding time fifty per cent or more of their root system was destroyed. Nevertheless a yield of 24 bushels to the acre was produced. Unquestionably this was due to the fact that the surface roots, which are more numerous in damp than in dry soil, were able to supply the plant with water. During the dry season of 1916 the writer observed numerous bean fields affected with the dry root-rot which did not produce over two or three pods to a plant. In this season there was sufficient moisture directly after planting time to cause a succulent growth in the plant and a rapid progress of the disease. Throughout the dry period that followed, the plants were checked in their growth and few pods were formed.

EFFECT OF DRY ROOT-ROT ON THE YIELD OF THE BEAN CROP

Few experiments have been conducted to determine the exact effect of any plant disease on the yield of its host. For a crop that is subject to numerous diseases, such information is frequently of extreme value. This is true of the bean, in the case of which several pathogenes may occur on the roots of the plants. One of these is the dry root-rot, and, since inoculations can be controlled, an experiment to learn the effect of this disease on the bean crop can readily be conducted.

Since the black root-rot is so frequently associated with the dry root-rot, it was considered advisable to determine the loss in yield resulting from each as well as from a combination of the two diseases. For the sake of accuracy such an experiment should be repeated through several seasons in order to avoid the effect of unusual weather conditions. A preliminary experiment of this type was conducted in 1916. The seed was not planted until July 20, and therefore the crop did not mature before the early frost of that season. Plants in the rows inoculated with *Fusarium martii phaseoli* were considerably dwarfed, but those inoculated with *Thielavia basicola* could not be distinguished from the check rows. A combination of the two, from all appearance, caused no more injury than did *F. martii phaseoli* alone. It was unfortunate that the beans could not be harvested, inasmuch as the weather was especially favorable for the effects of the disease to appear in the yield.

In 1917 a more extensive experiment was conducted at Ithaca. The weather conditions were very unfavorable for the dry root-rot, but were advantageous for the bean plant. Marked losses, therefore, which would be noted in average years or in years favorable for the disease, were not obtained. Some interesting facts appeared in the experiment, and for this reason the data, although incomplete, are set forth here.

The plot at Ithaca used for the experiment was located on a low piece of ground. The soil was a gravelly loam. In previous years the plot had been in alfalfa, and no records show that it had ever been planted to beans. It was likewise out of the bean area and the soil proved to be free from the pathogene *Fusarium martii phaseoli*. The soil was likewise free from *Thielavia basicola*, since all checks remained healthy even though this organism is widely distributed and occurs on such hosts as alfalfa.

The plan of the experiment was as follows: Plants in row 1 were inoculated with *Thielavia basicola*; row 2 remained as a check; row 3 was inoculated with *T. basicola* and *Fusarium martii phaseoli*; row 4 was a check; row 5 was inoculated with *F. martii phaseoli*; row 6 was a check. The rows were three feet apart and one hundred feet long. They were repeated five times. To eliminate outside rows, buffer rows were planted on either side of the plot.

The seed, which was a Marrow Pea variety, was planted on June 14. Rain had fallen on the preceding day and the soil contained sufficient moisture to keep the spores of the fungi from being killed by drying. The same quantity of seed was used for each row, and all seeds were inoculated with the legume bacteria before planting. The check rows were covered before the others were inoculated, to avoid any danger of contamination.

During the summer the plot was cultivated twice and weeded, but it was not hoed. Thus there was little danger of the fungi's spreading from the infected rows to the check rows. When the plants were pulled in the fall no infection was found to have occurred on the check plants.

Since the season was wet and the plots were located in the low ground, plenty of moisture was retained in the soil. After June 14 rain fell on the following dates: June 15, 18, 19, 20, 23, 26, 27, 28, and 29 — a total precipitation of 3.89 inches. During July rain fell on the following dates: July 1, 2, 3, 4, 6, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, 20, 21, 26, and 29 — a total precipitation of 3.25 inches. In the month of August rain fell on the following days: August 2, 3, 6, 8, 9, 10, 13, 14, 15, 16, 22, 23, 28, 29, and 30 — a total precipitation of 8.45 inches. In the first half of September there was precipitation on the 1st, 2d, 6th, 7th, 8th, 9th, and 10th, with a total of 1.41 inches. Thus it may be seen that the soil was in a moist condition throughout the summer, especially during the critical month of August when the pods are produced. The plants grew rapidly and soon covered the ground. The majority produced a large number of pods, and from the general appearance the inoculated rows could not be distinguished from the check rows.

When the plants were pulled in the fall, considerable injury was observed on the roots of the plants inoculated with the dry root-rot fungus. In many cases all that remained of the roots was a dried stub, but the parts above ground were apparently healthy. The rows affected with the black root-rot showed more injury than during the dry, hot summer of 1916. Few plants, however, showed serious injury. Those inoculated with both the fungi could not be separated from those inoculated with *Fusarium martii phaseoli* alone. As to the yield in the different rows, nothing could be determined from observation. Each row was harvested and threshed separately and the amount of seed was weighed. The data are given in table 3:

TABLE 3. EFFECT OF ROOT DISEASES ON THE YIELD OF THE BEAN DURING THE SEASON OF 1917

Row	Inoculation	Weight of seed (grams)	Reduced or increased yield (per cent)*
	Buffer row		
1	Black root-rot	3,585	72.6
2	Check	4,938	
3	Dry root-rot Black root-rot	3,749	76.4
4	Check	4,877	
5	Dry root-rot	4,743	105.9
6	Check	4,083	
7	Black root-rot	4,843	107.4
8	Check	4,933	
9	Dry root-rot Black root-rot	3,538	72.9
10	Check	4,778	
11	Dry root-rot	3,751	81.0
12	Check	4,483	
13	Black root-rot	4,722	100.5
14	Check	4,916	
15	Dry root-rot Black root-rot	3,759	75.8
16	Check	5,006	
17	Dry root-rot	4,781	96.3
18	Check	4,922	
19	Black root-rot	4,565	101.1
20	Check	4,111	
21	Dry root-rot Black root-rot	3,915	92.6
22	Check	4,345	
23	Dry root-rot	4,242	105.0
24	Check	3,738	
25	Black root-rot	2,421	58.0
26	Check	4,604	
27	Dry root-rot Black root-rot	2,810	65.0
28	Check	4,041	
29	Dry root-rot	3,394	82.7
30	Check	4,162	
	Buffer row		

* The reduced or increased yield is calculated by determining the percentage of yield of the diseased row in relation to the average of the yields of the two adjacent check rows.

In studying this table several points should be taken into consideration which have or might have a disturbing effect on a proper interpretation of the data. In all instances these points deal with individual rows. Since the first row served as a buffer, the yield was not determined, and hence the check percentage of row 1 was calculated from only one check row. In all other cases the average of the check rows on both sides of the diseased row was used. In check row 6 it may be seen that the yield was far below the normal. The cause of this is unknown. It resulted, however, in unfair calculated check percentages for rows 5 and 7. If rows 4 and 8 only had been used, the check percentages would not have been above one hundred. Row 25, which shows a very low yield and therefore a low check percentage, had a small number of plants. Row 27 also had a small number of plants, but this was not evident on the yield. The writer appreciates the fact that rows of equal length may have varying numbers of plants without affecting the yield of these rows. For this reason it is considered best to use rows in an experiment of this type instead of a certain number of plants. Just how much thinning a row may permit before a noticeable decrease in yield occurs is not known.

In consideration of these disturbing factors, very few data are presented which show that the black root-rot reduces the yield of beans to any extent. The weather, no doubt, was as favorable as possible for this disease and the root-rot approached its maximum in severity. The injury from the black root-rot was brought about to a large extent by the destruction of the plants in the seedling stage. The remaining plants, therefore, had a better opportunity to develop and offset this injury. The calculated check percentages for the dry root-rot on the whole are high. This is to be expected, however, when the soil retains a large amount of moisture throughout the growing season. With the combination of the diseases there appears to be a consistent reduction in the yield.

A further experiment to determine the effect of the dry root-rot on the yield of the bean was conducted during the season of 1918. The plot of land selected, although not so advantageous for beans, was located on higher ground than that used in the experiment of 1917. The soil contained a considerable amount of clay. The plan of the experiment was the same as in 1917 with the exception that only the fungus *Fusarium* *martii phaseoli* was used. Six rows were inoculated, with a check row left between each two treated rows. All seed used in the experiment was inoculated with the root-nodule organism.

During the summer the plot was cultivated twice and weeded. At podding time a slight difference could be distinguished between the check rows and the rows of infected plants. That this should occur in 1918 and not in 1917 was due to the lower ground on which the plot was located in 1917 and to the general raininess and cloudiness of that growing season. The plot was planted on June 4, after which rain fell on the following dates: June 6, 7, 10, 11, 12, 13, 14, 21, 22, 23, 25, 28, and 29 — a total precipitation of 3.05 inches. Thus a sufficient amount of soil moisture was present during the infection period. In July rain fell on the following dates: July 1, 3, 5, 9, 10, 11, 17, 24, 29, and 30 — a total precipitation of 4.55 inches. During August, when the pods are formed, there was less than half as much precipitation as in the month of August, 1917; rain fell on the following dates: August 4, 5, 8, 10, 14, 24, 26, 28, 29, and 30 — a total precipitation of 3.76 inches. The plants were harvested in the first part of September. The results of this experiment are shown in table 4:

TABLE 4. EFFECT OF THE DRY ROOT-ROT ON THE YIELD OF THE BEAN DURING THE SEASON OF 1918

Row	Inoculation	Weight of seed (grams)	Reduced yield (per cent)*
1	Check	291	
2	Dry root-rot	548	61.9
3	Check	1,478	
4	Dry root-rot	723	47.9
5	Check	1,538	
6	Dry root-rot	954	56.3
7	Check	1,848	
8	Dry root-rot	709	43.8
9	Check	1,392	
10	Dry root-rot	720	61.7
11	Check	941	
12	Dry root-rot	484	51.4

* The reduced yield was calculated as in table 3 (page 1024).

The experimental plot used in 1918 was an apparently level piece of ground, nevertheless it may be seen that the yield near the center was much higher than that on either side. However, observation in most bean fields will show that the outside rows are composed of low-yielding plants. In other respects the data for this experiment are not difficult to analyze. Weather conditions were such as to make the effect of the dry root-rot very noticeable. The decrease in yield in diseased rows averages approximately fifty per cent.

CONTROL

ROTATION OF CROPS

Since the cause of the dry root-rot is a soil pathogene, the possibility of controlling the disease through longer crop rotations was considered very early in these investigations. The common rotation practiced by the growers of dry shell beans is but three years, and frequent instances are found in which the rotation is only two years. It is customary to follow beans with wheat and then clover, although in the fruit-growing districts this rotation may vary somewhat. In all localities, however, a few growers vary their rotations considerably. For this reason a survey was made in Wyoming County in 1915, and in Genesee, Livingston, and Monroe Counties in 1916, to determine whether these variations in rotation affect the disease to any extent. Very little information was obtained. Fields were observed in which beans had not been grown for ten years and yet the dry root-rot was present. In some of these fields the pathogene might have been continuously returned to the soil in the manure. On the other hand, this explanation could scarcely hold for pasture land.

One point drawn from the survey that should be noted is, the shorter the rotation the more serious is the disease, although long rotations do not eradicate the fungus. From this it appears that the pathogene will accumulate more rapidly in the presence of the bean plant. Nevertheless it still persists in the absence of this host plant, and, as far as is known, no other crop grown in rotation with the bean and no weeds found commonly in the fields are susceptible to its attacks. It would thus appear that the fungus lives saprophytically in the soil. It is not uncommon for pathogenic members of the genus Fusarium to do this, since they frequently have been isolated from the soil in the absence of their host plant. Pratt (1918) isolated *Fusarium radicola* Wollenw. and *F. trichothecioides* Wollenw., two potato pathogenes, from virgin soil in Idaho.

From the foregoing observations on the character of *Fusarium martii phaseoli*, it appears highly improbable that the dry root-rot of beans can be entirely eliminated by longer crop rotations.

SOIL TREATMENT

The application of chemicals and fertilizers to the soil to destroy or hold in check soil pathogenes, has seldom proved successful on an extensive scale. In seedbeds and greenhouses where expense does not prohibit, treatment with formaldehyde may be used profitably. A formaldehyde solution applied in the furrow with the seed at the time of sowing has proved effective in the control of onion smut. On the other hand, the application of fertilizers has tended at times to lessen the effect of certain root diseases. Such has been the case in liming fields contaminated with the organism causing clubroot of cabbage. Acid phosphate applied to tobacco fields also has proved to be beneficial in holding in check the black root-rot.

During the season of 1915 a number of farmers near Perry and Warsaw, New York, under the direction of the Wyoming County Farm Bureau tried varying applications of lime and acid phosphate on their bean fields. Four of the farms were visited by the writer with H. M. Bowen, the County Agent, and careful observations were made. In no instance could it be determined that the fertilizers checked the disease to any extent. Similar reports were received from various other farms.

The application of toxic substances to the soil, especially non-volatile substances, must be made with great care. The roots of the plants are extremely sensitive and in the seedling stage great injury may be done to them. The pathogene, too, is frequently very resistant to chemicals and cannot be destroyed easily. Furthermore the after effects of treatment with various chemicals have been reported to be injurious to other crops grown in rotation. Sherbakoff (1915 a) has shown this to be true for clover when sulfur was applied previously; and Wheeler, Hartwell, and Moore (1899) have shown the same thing to be true for cereals.

However, regardless of the fact that clover generally occurs in the rotation with beans, a preliminary test was made with sulfur in the summer of 1915. Sulfur was drilled in with the seed at the rates of 200 pounds and 400 pounds to the acre. In neither case was the germination of the seed injured, but over 95 per cent of the plants showed severe infections of the dry root-rot. Later in the summer these rows were torn up and again planted to beans. It was thought that by that time the sulfur might have had an effect on the pathogene. Practically all the plants became infected. No further experiments with sulfur were conducted.

A volatile chemical which would kill the pathogene and then evaporate would be the ideal substance to apply. The use of formaldehyde possibly furnishes the closest approach to this condition. Calcium hypochlorite is another substance which would break down and leave no injurious substances. In the winter of 1915–16 preliminary tests were conducted with these two substances. One of the beds in the greenhouse was thoroughly contaminated with *Fusarium martii phaseoli*. It was then divided into three plots, each containing 12 square feet, and planted to beans. Plot 1 was treated with calcium hypochlorite applied at the rate of 200 pounds to the acre and drilled in with the seed; plot 2 was left as a check; plot 3 was treated with a formaldehyde drip similar to that used in onion smut treatment. The strength of the formaldehyde solution used was 1:100 and it was applied at the rate of 400 gallons to the acre. The germination of the seed was not injured by the formaldehyde, but injury to the extent of 75 per cent was observed on the plot treated with calcium hypochlorite. In both plots all plants were as severely infected as those in the check plot.

Further experiments with calcium hypochlorite and formaldehyde were conducted in the field in the season of 1916. A bean field near Perry, known to be contaminated with *F. martii phaseoli*, was used for the purpose. In each plot the beans were planted in four rows 28 inches apart, each row containing about 100 plants. Some of these were destroyed by the seed-corn maggot and by slugs. Plot 1 was treated with calcium hypochlorite at the rate of 200 pounds to the acre, the treatment being made ten days before planting; plot 2 was treated with the same substance at the rate of 100 pounds to the acre, and the chemical was drilled in at the time of planting; plot 3 was treated with formaldehyde, 1:100, which was applied as a drip at the time of planting at the rate of 400 gallons to the acre (this plot was repeated three times); in plot 4 the strength of the formaldehyde was doubled. Only in the last plot was any injury to the germination of the seed observed. No beneficial effects were noted. In all plots, including the check, the proportion of healthy plants did not exceed 5 per cent.

Similar experiments were conducted in 1918 with the commercial preparation cyanamid. Three plots, each 20 feet long and 12 feet wide, were treated with varying amounts of the chemical, and a plot of similar size was used as a check. Plot 1 was treated at the rate of 50 pounds to the acre, plot 2 at 75 pounds to the acre, and plot 3 at 100 pounds to the acre. The applications were made at the time of planting, which was August 10, and the chemical was drilled in the rows. The variety of beans used was Well's Red Kidney. This method of treatment injured the germination considerably and retarded the growth of the plants to a severe extent. The experimental plots were examined on October 3. Over 90 per cent of the plants in the treated plots showed severe infection of the dry root-rot. No distinction could be made between these and the plants in the check plot. The results do not warrant further experiments with cyanamid.

USE OF BEANS RESISTANT TO ROOT-ROT

Very little progress has been made in controlling plant diseases caused by soil-inhabiting fungi, except by the selection and development of disease-resistant strains. This is especially true when the pathogene concerned belongs to the genus Fusarium. Orton (1908) has used this method in checking the wilt of cotton, cowpea, and watermelon. Bolley (1903), and more recently Tisdale (1917 b), have selected wilt-resistant varieties of flax. Jones and Gilman (1915) have employed the method with great success in controlling the yellows of cabbage. Norton (1914) and Edgerton (1918) have selected tomatoes resistant to the Fusarium wilt.

In New York State, fields of the White Marrow bean are frequently lacking in uniformity of type of the bean. Among the varying types is a strain to which, for convenience, the name *Flat Marrow* has been given by the writer. During the summer of 1915 it was observed that this strain exhibited a high degree of resistance to the dry root-rot.

In character the Flat Marrow varies somewhat, from a true White Marrow to a Burlingame (or Medium, as it is commonly called in New York State). The plant has a compact form of growth with broad and dark green leaves. The stems are heavier than in the common Marrow. The shape of the pod is that of a Medium, while frequent dashes of blue lead to the belief that the strain is closely related to the Blue Pod Medium. The seeds are smaller and flatter than in the Marrow, but seldom are they the shape of those of the Burlingame. During the early part of the season these plants are difficult to distinguish from the Marrow, but a few weeks before harvest time they may readily be distinguished from the other varieties. This is due practically to the fact that the resistance to root-rot is remarkably noticeable (Plate LVI, 3). The Flat Marrow cannot be grown commercially in New York State, however, because of several objectionable characters. It is later than the White Marrow and, altho very productive, it frequently is injured by frosts. Another undesirable character is the length of its podding season. When the first pods are ripe the later ones are still small and green. Nevertheless its character of resistance to root-rot makes it a valuable bean.

It has been shown by the writer (1918) that resistance to anthracnose in beans can be transferred from one variety to another by hybridization. It was therefore hoped that by crossing the Flat Marrow with any of the common commercial varieties of bean, strains of the latter might be obtained resistant to the dry root-rot. In making selections among the Flat Marrow for parent stock, other characteristics besides disease resistance were considered. These were, earliness of maturing, and tendencies toward a White Marrow or a Medium type of bean. In 1915 only nineteen individuals were selected. The progeny of these were tested for resistance in the greenhouse during the following winter and in the field during the seasons of 1916 and 1917. Only seven strains were discarded. Numerous crosses have been made by the writer between the Flat Marrow and the White Marrow, and more recently E. W. Lindstrom and G. P. McRostie have hybridized the resistant type with a number of other commercial varieties of dry shell beans. The F_2 generation of all the first hybrids have been grown, and part of the F_3 generation. These have exhibited a very complex segregation, and it is the opinion of the writer that the character of disease resistance in this case is not governed by simple factors.

As yet no White Marrow strain has been isolated resistant to the dry root-rot. Insufficient knowledge concerning the genetical behavior of the characters of resistance to this disease, however, renders it impossible to state with certainty what may be done. Nevertheless it is hoped that desirable types possessing root-rot resistance may be obtained from the above crosses.

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BEAN ANTHRACNOSE

MORTIER F. BARRUS

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BEAN ANTHRACNOSE

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MORTIER F. BARRUS

THE HOST

Next to the potato and the sweet potato, the bean (considered as a vegetable) is the most important crop grown in this country. Not only are beans grown in practically every home garden, but they are of great commercial importance also. The United States Census Bureau (1913a) reports the production of dry edible beans grown for commercial purposes in this country during 1909 as 11,251,160 bushels, or more than double that of 1899, representing a value of $21,771,482. The Monthly Crop Report of the United States Department of Agriculture (1917: 133) estimates the production for 1916 as 12,029,000 bushels; for 1917 it was 18,129,000 bushels, the culture of beans having been stimulated by the demand and by the high prices paid as a result of the war. Since the war there has been a marked reduction in area in the States having a high bean production. Dry beans are a staple crop in New York, Michigan, Colorado, New Mexico, California, and several other States, while string beans are grown extensively in many parts of the country as a truck crop. In northern New York, in Michigan, Colorado, and California, and in southern Canada, the production of garden and field beans for seed has developed into a large and profitable industry. Large areas also are devoted to the production of snap beans for canning.

Prior to 1899, New York was the leading State in the production of dry edible beans, in 1879 producing 42.4 per cent of the total crop in the United States and in 1889 producing 35.1 per cent. In 1909, however, it produced only about 15 per cent of the total crop (U. S. Census Bureau, 1913a). While production has greatly increased in the other large bean-producing States (table 1), New York has lagged behind, although it was stirred on somewhat during 1917 and 1918.[1] Low yields resulting from disease and from poor weather conditions inevitably resulted in reduced acreage for 1919.

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[1] Data for 1879 and 1889 were obtained from the Eleventh Census; for 1899 and 1909, from the Thirteenth Census; for 1914, from the Monthly Crop Report for 1916; for 1915, 1916, and 1917, from the Monthly Crop Report for 1917; for 1918, from the Monthly Crop Report for 1918; for 1919, from the Monthly Crop Reporter for 1919; for 1920, from the Monthly Crop Reporter for 1920. These references are given under *Literature Cited*, page 199.

TABLE 1. ACREAGE AND PRODUCTION (IN BUSHELS) SINCE 1879, OF DRY EDIBLE BEANS IN THE FIVE LARGEST BEAN-PRODUCING STATES AND IN THE UNITED STATES

Year		New York	Michigan	California	Colorado	New Mexico	United States
1879	Production...	1,303,444	167,658	378,971	....	16,168	3,075,050
1889	Production...	1,111,510	434,014	713,480	7,265	7,843	3,163,554
1899	Acreage......	129,298	167,025	45,461	2,406	17,417	453,841
	Production...	1,360,445	1,806,413	658,515	28,570	36,022	5,064,490
1909	Acreage......	115,698	403,669	157,987	5,040	20,766	802,991
	Production...	1,681,506	5,282,511	3,328,218	53,926	85,795	11,251,160
1914	Acreage......	118,000	490,000	215,000	20,000	32,000	....
	Production...	1,650,000	5,488,000	3,875,000	300,000	272,000	....
1915	Acreage......	130,000	506,000	225,000	21,000	46,000	....
	Production...	1,495,000	4,250,000	3,868,000	340,000	368,000	....
1916	Acreage......	190,000	470,000	340,000	38,000	64,000	1,244,000
	Production...	1,140,000	3,102,000	5,576,000	424,000	425,000	12,029,000
1917	Acreage......	210,000	639,000	558,000	193,000	213,000	2,117,200
	Production...	1,575,000	3,514,000	8,035,000	1,467,000	958,000	18,129,000
1918	Acreage......	200,000	543,000	592,000	252,000	149,000	....
	Production...	1,660,000	4,887,000	8,880,000	1,638,000	596,000	....
1919	Acreage......	90,000	300,000	400,000	69,000	123,000	....
	Production...	1,305,000	4,140,000	5,000,000	448,000	922,000	....
1920	Acreage......	90,000	275,000	285,000	63,000	121,000	....
	Production...	1,260,000	3,575,000	2,850,000	504,000	811,000	....

The figures in table 2, taken from statistics compiled by order of the New York State Food Commission (1919), give the acreage and production during 1917 of important bean-growing counties in the State and show the location of the bean area. A larger yield to the acre was obtained by some of the other counties which planted a smaller acreage.

TABLE 2. ACREAGE AND PRODUCTION OF FIELD BEANS IN THE TEN LARGEST-PRODUCING COUNTIES OF NEW YORK STATE IN 1917

County	Acreage	Production (bushels)
Livingston	37,010	325,245
Wyoming	23,146	200,949
Genesee	18,835	101,158
Ontario	13,884	82,353
Wayne	12,615	86,105
Monroe	11,076	42,751
Orleans	9,640	38,561
Erie	9,235	46,547
Yates	9,007	70,442
Schuyler	8,164	59,650
New York State	218,742	1,477,061

New York ranks first of all the States in the amount and value of canned string beans, the United States Census Bureau (1913b) reporting 452,634 cases having a value of $839,135, or more than half the total value for the entire country. It is assumed that nearly all string beans canned in the States were grown within its territory.

It is thus seen that the bean industry in New York and in the country at large is an important one, and well worthy any attention that may be given to it by the Federal Government and by the various experiment stations.

The common bean (*Phaseolus vulgaris* L.) becomes affected at times by various diseases, among which the bean anthracnose is an important one in New York State. Bacterial blight also occurs frequently on the common bean and on the lima bean (*P. lunatus* L.), and was severe in 1916 and 1917. Root rots — especially the one caused by *Fusarium Martii phaseoli*, though black root rot, caused by *Thielavia basicola* Zopf., should be mentioned also — are causing considerable loss in restricted localities and may be more prevalent than is generally known. Rhizoctonia stem canker and pod spot, caused by *Corticium vagum* B. & C., has been reported from a few places as being serious and is known to have a general distribution. Bean rust, caused by *Uromyces appendiculatus* (Pers.) Lév., occurs occasionally, but does little damage in this State though it is severe at times in the South and the West. Brown rot, caused by *Sclerotinia libertiana* Fuckel, is destructive only during periods of extended rains and warm weather, and then only on vines so bushy that they do not permit good aëration. The leaf spot caused by *Phyllosticta* sp. is uncommon in the State, while leaf spot caused by *Isariopsis griseola* Sacc. has not been reported here.

In addition to the parasitic diseases just mentioned, mosaic, supposed to be due to a filterable virus, should certainly be included because of its general prevalence in the State and its apparently serious effect.

The writer has devoted more or less of his time for the past ten years to the study of the anthracnose disease and the manner of its control. In the following pages he presents his opinions and those of others in regard to the disease, and records the results of his experiments.

THE DISEASE

ECONOMIC ASPECTS

Name, history, and geographical distribution

The name *bean anthracnose*, generally applied by American, English, French, Italian, and other pathologists to the disease under consideration, was apparently first used in this connection by Scribner (1888), although the name *anthracnose* was used earlier to designate the grape disease caused by *Gloeosporium ampelophagum* Sacc. The terms *pod spot*, or *canker*, and *leaf spot* are sometimes applied to the disease as it occurs on these respective parts of the plant; while not uncommonly the terms *rust* and *blight*, and rarely *scab*, are erroneously used by laymen. Frank (1883 b) called the disease *Fleckenkrankheit*, and the names *Brennerflecken*, *Brennerfleckenkrankheit*, *Brennfleckenkrankheit*, and *Schwarzfleckenkrankheit* have been used by German writers. In Holland, Schenk (1917) speaks of the disease as *Vlekziekte*, and in Denmark it is called *Bonnesyge* by Lind (1910).

The disease was first definitely described by Saccardo (1878), who reported its discovery by Lindemuth in the fruit and vegetable garden of the Agricultural Institute of Poppelsdorf, at Bonn, in August, 1875. Anthracnose spots are present, however, on specimens of bean pods distributed by Desmazières (1843 b) under the name *Septoria leguminum* Nob. Berkeley (1880) reports the disease on kidney beans from Bedford, England, ascribing it to Ascochyta. Saccardo (1881) shows a good colored reproduction of a pod affected with anthracnose, and drawings of the fructifications of the fungus. The label indicates that the fungus was collected at Padua in June, 1875, on *Phaseolus vulgaris*. Frank (1883 b) states that Lindemuth reported to him the occurrence of the disease in 1875 on the red-mottled Zucker-Stangenbrechbohnen, and a few years later on numerous other varieties grown in the same vicinity. Frank himself says the disease occurred in other parts of Germany in 1881 and in various localities in 1882, it being especially severe the latter year. He gives an excellent account of the disease and its cause in the article cited. Saccardo (1884) mentions the disease as being common and injurious in Germany, Italy, France, England, and North America. Richon (1889) records its presence in the Marne district of France.

Ellis and Everhart (1885:111) report Dr. Farlow's observations of the common occurrence of this fungus on beans in the Cambridge (Massachusetts) market since 1882. From the Plant Disease Bulletin issued by the Plant Disease Survey of the United States Plant Industry Bureau (1918:254), it appears that the disease was reported from Pennsylvania, Maryland, and Louisiana in 1887; from Michigan in 1889; and from New York and Ohio in 1890. Scribner (1888:361), after speaking of its distribution in Europe, says that specimens have been received from Maine, Massachusetts, Wisconsin, Pennsylvania, Louisiana, and the District of Columbia.

Since then the disease has been reported from a number of places in Germany, Italy, England, and the United States. Reports of its existence — some recording only its presence and others its prevalence and destructiveness — have been recorded from Norway (Schoyen, 1901), Sweden (Eriksson, 1889), Denmark (Lind, 1910), Russia (Jaczewski, 1912), Tyrol (Bubák and Kabát, 1904), Belgium (Marchal, 1907), Holland (Sprenger, 1918), Ireland (Schoebotham, 1909), Japan (Ideta, 1911), Formosa (Fujikuro, 1914), India (Butler, 1918), Transvaal (Pole-Evans, 1907), Australia (Cooke, 1892), New Zealand (Kirk, 1905), Argentina (Spegazzini, 1899), Brazil (Puttemans, 1901), Cuba (Cook and Horne, 1908), Alaska (Anderson, 1916), and Canada (Craig, 1893).

From the bulletin issued by the Plant Disease Survey (U. S. Plant Industry Bureau, 1918) and from the earlier survey reports by Orton and by Orton and Ames (1904 1909), as well as from the bulletins and reports of the various experiment stations in this country, it appears that the disease has occurred at one time or another in every State in the Union except South Dakota, Wyoming, and Nevada. Doubtless it has occurred in these three States also but has not been reported.

It is thus seen that the disease is world-wide in its distribution, occurring in nearly every place where beans are grown. The climatic condition of a country or a region has much to do with the development of the disease, so that in some of the countries where it has been reported it is of no importance and in others it becomes a serious pest. While it has been reported from Alaska, it cannot be enphytotic there, as seed of *Phaseolus vulgaris* will not mature in that latitude; but the summer climate of Alaska is not unfavorable for the development of the fungus when introduced in imported seed affected by it.

Economic importance

While bean anthracnose can be found on some plants year after year, it is only during certain periods most favorable for its development that it becomes widespread and causes serious loss. Such epiphytotics are coincident with or closely follow periods of rather abundant rainfall during the growing season, and often recur in three or more successive years. Frank (1883 b:512), as already noted, reports such an epiphytotic in Germany in 1882, Querner (1908) reports one in 1908, and Fischer (1919:246) reports epiphytotics in 1915 and 1916. Voglino (1892) reports a complete destruction of the crop in 1891 in several localities in Italy.

Beach (1892:308) reports a heavy loss in many sections of New York in 1891. Halsted (1893–1901) reports the disease as common from 1892 to 1897, after which it was an unimportant factor in his experiments. Whetzel (1908:436) reports the disease as widespread in New York in 1906. Then followed five years during which the loss in most sections of the State was negligible. Late in the season of 1911, the disease was observed or reported from many fields, and in 1912 it was again severe. The following year there was very little anthracnose to be found except in restricted areas, but it was present to a destructive degree in 1914 and 1915, the loss during the latter year ranging from 30 to 100 per cent of the crop except in a few cases where resistant varieties were grown. During 1916 the damage done in this State was slight, but the disease appeared again from 1917 to 1919, the losses in these years, however, being much less than in earlier years.

A study of the table given in the Plant Disease Survey bulletin (page 254 of the reference already cited) shows that bean anthracnose was generally severe in the eastern United States from 1906 to 1908, from 1914 to 1915, and again in 1917. Individual reports of its severity are recorded in other years. In Maine and New Hampshire it is reported as causing considerable loss nearly every year, while in Western States it rarely does any damage although it may be present.

Figures are not obtainable to show the exact loss from this disease, but in epiphytotic years it is very large, amounting in individual cases to 100 per cent. Muncie (1917:7) estimates a loss in Michigan of $1,500,000 in 1914 and of about $3,000,000 in 1915, while the loss in New York in 1915 probably amounted to $700,000 or more. The Plant Disease Survey bulletin (page 13 of reference cited) gives the loss during 1917 for New York as 5 per cent, or 83,000 bushels, and for the United States as 1.76 per cent, or 363,000 bushels.

The losses from bean anthracnose are due to poor germination of affected seed, to destruction of affected seedlings, to low yield of affected plants, and to decreased value of the product. The principal loss in the production of green beans is due to the spotting of the pods, which renders them unsalable as snap beans and unfit for canning. Southern-grown beans, apparently healthy when shipped, frequently reach northern markets in a badly spotted condition, and if the disease is common in the field, pods kept over night after picking are likely to be rusted the next morning. The lesion may extend through the pod to the seed, discoloring it. The value of dry seed thus affected is lessened, and the buyer usually deducts a certain percentage, the "pick," from the total weight to allow for this depreciation. A poor stand often results from the planting of spotted seeds, many of which fail to germinate, and this result is augmented by the destruction of seedlings subsequently affected. Plants that have survived the early attack may give an inferior yield due to interrupted growth, although the pods themselves may not become spotted.

SYMPTOMATIC ASPECTS

While the appearance of anthracnose on the various parts of the bean plant has been described accurately by several investigators, a description is included here of its appearance as observed on different varieties of beans under different conditions and at different stages of maturity.

On the seed

The seed of the bean may be discolored by anthracnose, blight, rhizoctoniose, and brown rot, and by various mold-producing fungi. The appearance most characteristic of anthracnose (Plate IV) is the decided blackening of the affected parts. Usually the blackened area has a tawny brown or tan-colored[2] border, or the entire diseased area may consist of the latter color. Specimens are common in which the color of the spot on the seed is lighter, varying from drab to snuff brown or saccardo umber. The larger spots usually have a vinaceous buff to avellaneous-colored, often raised, center, which sometimes splits open disclosing the affected cotyledon. The spots may occur on any part of the seed coat, and may appear as a small black or brown speck or involve more than half the surface. In badly diseased seeds the lesion extends through the cotyledon and may even affect the embryo, but in many cases the cotyledon is but slightly or not at all discolored.

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[2] The colors as given correspond to those given in Ridgway's *Color Standards and Nomenclature*, and, while characteristic, there is considerable variation from them.

On the seedlings

Seedlings from badly diseased seed show blackened cankers on the cotyledons (fig. 10), in which, under moist conditions, small flesh-colored masses appear scattered over the surface of the lesions. The stem may become affected along its entire length, but usually it is affected only below the point of the attachment of the cotyledons. The first indication of a lesion is the appearance of brown specks in the epidermis. Later there appears a browning of the tissues lengthwise of the stem and below the epidermis. Still later, small pits appear along the affected area. Occasionally these cankers become so deep or so numerous that the stem is unable to support the parts above.

On the leaves

The leaves (figs. 10 and 11) not infrequently become badly affected. The lesions appear on the veins, usually on the underside of the leaf. These lesions have practically the same appearance as those on the stem. The tissues on each side of an infected vein and beyond it may wither and die. A considerable area of a leaf may become affected in this manner, giving it somewhat the appearance of blight. Usually only a small part of the leaf tissue is killed, and such places appear on the upper side as angular, elongated, dead areas, which sometimes become torn, giving the leaf a ragged appearance. The petiole may become so badly affected that it cannot support the leaf blade.

On the roots and other parts

The roots of plants growing in the soil are rarely if ever affected; but on seeds sprouted in a moist chamber, where the young roots are exposed, lesions similar to those on the stem may be produced by artificial inoculation. Muncie (1914: 4) mentions a rotting of the root due primarily to the anthracnose and blight organisms followed by secondary rot-producers

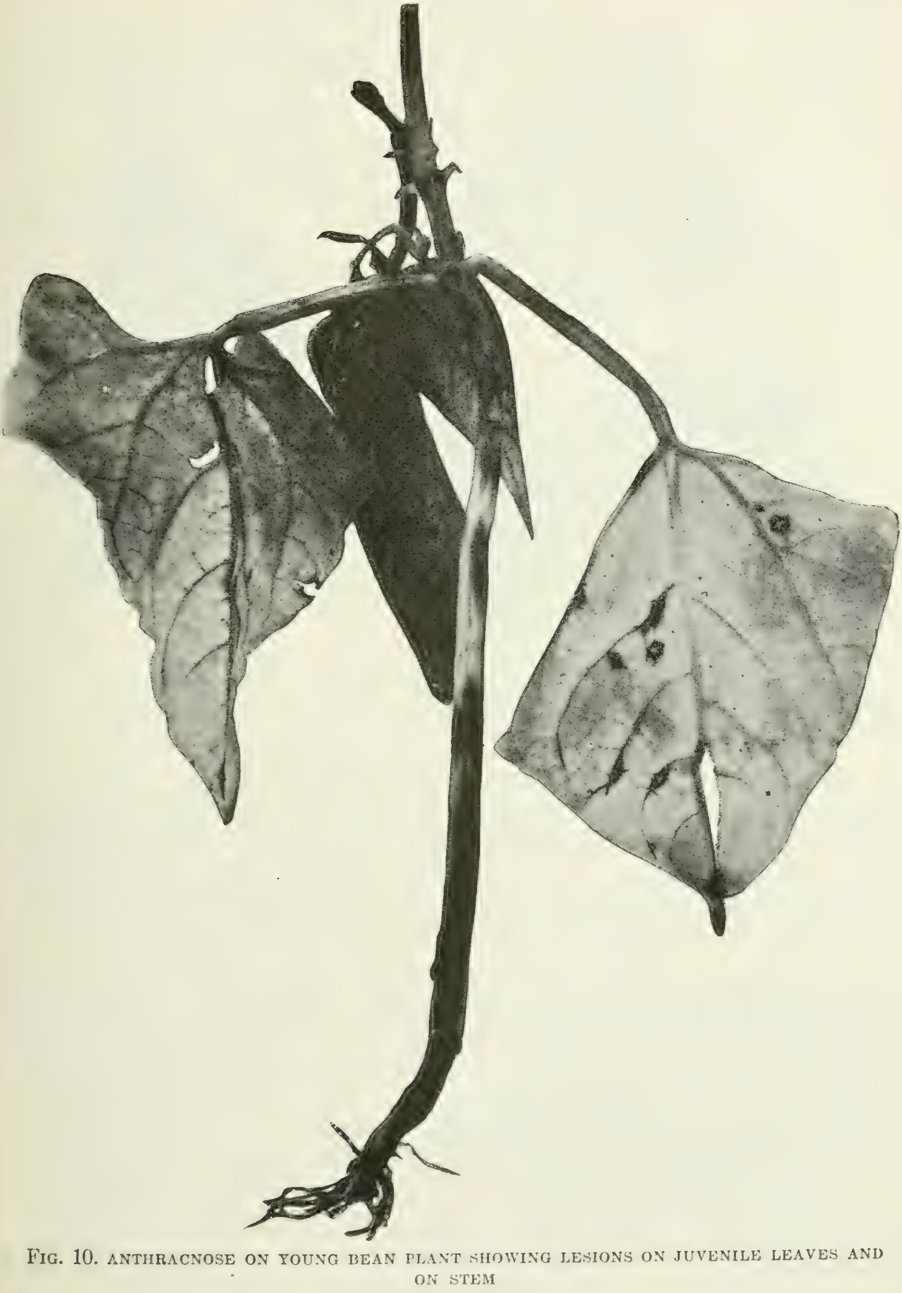

FIG. 10. ANTHRACNOSE ON YOUNG BEAN PLANT SHOWING LESIONS ON JUVENILE LEAVES AND ON STEM
(Photographed by H. H. Whetzel)

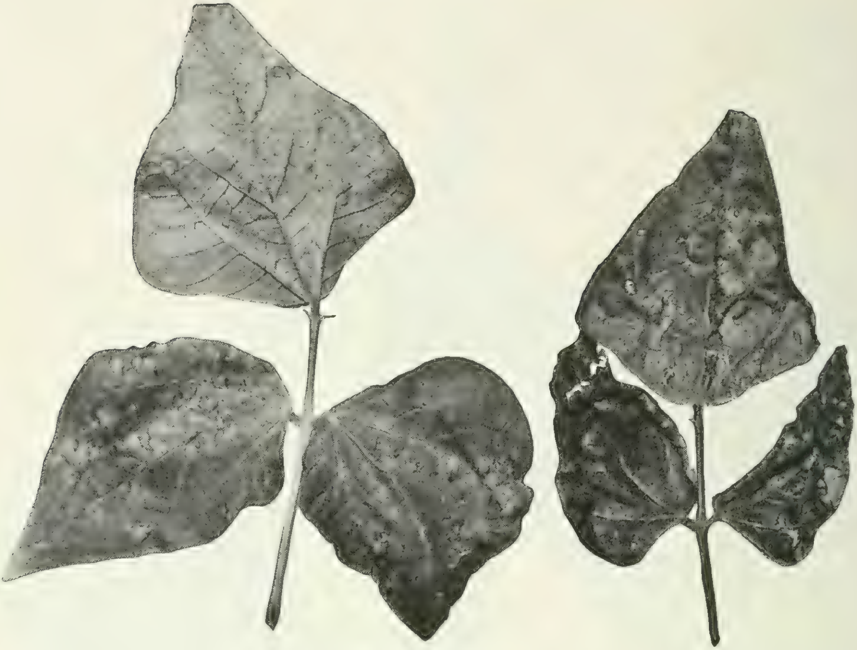

FIG. 11. ANTHRACNOSE ON BEAN LEAVES

Lesions on the veins cause death of the mesophyll about them, and show as brown dead spots or irregular areas on the upper surface of the leaves

The other parts of older plants may become diseased, and lesions similar to those already described may appear on the branches of the plant and on the pedicels, the sepals, and the bracts of the inflorescence. These parts of plants grown out-of-doors, however, become affected only during protracted periods of weather favorable for the organism.

On the pods

It is on the pods that the disease assumes its most striking appearance (Plate V). The first evidence of it there is the occurrence of tiny brown specks in the epidermis. These may be rufous or ferruginous, slightly elongated areas extending at an angle to the long axis of the pod. These specks enlarge to spots which become black at the center, with a vinaceous-rufous to hazel-colored border of varying width. These black spots with colored borders are characteristic of anthracnose. The cankers range in size from a mere speck to spots one centimeter in diameter or even larger. By the union of several large cankers, a broad lesion is often formed which may extend from one end of the pod to the other. A canker may extend through the endocarp and even to the seed, particularly if infection takes place early, in which case the pod sometimes fails to develop and becomes shriveled and dried. Cankers resulting from infections that occurred during the later growth of the pod seldom extend below the endocarp. As the pod matures, the lesion is marked at the edge of a canker by a slightly raised, black ring with a cinnamon-rufous to chestnut-colored border. The center of the spot is then somewhat light buff in color. Flesh-colored spore masses on the surface of a young canker dry down to gray, brown, or even black granulations or to small pimples.

The appearance of the disease on the pods of the different varieties is very similar. The lesions on Blue Pod Butter, a variety producing dark blue-purple pods, are snuff brown instead of black in color; otherwise they are similar to those on other varieties. On a number of picked pods that had been left in a basket and were somewhat wilted, and in other cases in which inoculations were made, lesions one centimeter or more in diameter appeared which were light brown in color and marked by a slight depression. If the spores had not been present one would hardly have believed the lesions to be anthracnose, particularly as the typical lesions were present also on some of the pods. In some instances these unusual-appearing lesions later assumed the more characteristic appearance. Lochhead (1903) mentions the presence of a pink rot (*Cephalothecium roseum*) in anthracnose spots on bean pods, and at Ithaca this fungus has been found often in such lesions.

Blight lesions (Plate VI) on the pods may sometimes be confused with anthracnose. The blight lesion consists of an area having a water-soaked appearance extending along the dorsal suture, or of spots of a similar nature and of various diameters appearing on the sides of a pod. Often a pod may have many such discolored spots, these having originated from insect bites or punctures. The spots usually have an ochraceous-tawny center with a border of a cinnamon-rufous color, and commonly, as the pod matures, the entire lesion turns to hazel or to cinnamon brown with a more highly-colored margin. It may be distinguished from anthracnose by the absence of any dark color,[3] by the shape of the lesions which are usually more extensive and more irregular in outline than those of anthracnose, and by the absence of spore masses.

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[3] A saprophytic Alternaria may occur on blighted leaves and pods and give a blackened color to the lesion.

Rhizoctonia (Plate VII) often occurs on the pod, producing lesions which, particularly when they are small, closely resemble anthracnose. This fungus attacks only the pods lying in contact with the ground. The lesion has a bay to auburn center, often with a dark-colored ring near the margin surrounded by a ferruginous border. The lesions are more extensive than those of anthracnose, however, and do not contain the characteristic spore masses (Plate I).

Sclerotinia libertiana Fuckel produces a disease characterized by a soft brown rot and moldy appearance of the pod, followed by a shriveling and drying, which can never be confused with anthracnose. This fungus commonly produces rather large black sclerotia on the pods.

On pods of certain varieties, rust (Plate VIII) sometimes occurs as pustules containing a black mass of dusty spores.

When immature pods are exposed to the hot sun — as is often the case when the leaves are injured by blight or by other means, especially when the plants are approaching maturity — the exposed surfaces become discolored with brown specks, spots, or blotches, in some cases a little sunken, which may be mistaken for blight but hardly for anthracnose. MacMillan (1918) describes these lesions as he found them on certain varieties of beans in Colorado and in the East, and the writer has observed them commonly in the East on many varieties. A peculiar browning, different from sun-scald, was also observed, but the cause was not determined. A browning of pods may occur from other causes also, none of which, however, need be mistaken for anthracnose.

ETIOLOGIC ASPECTS

Name, classification, and synonymy of the causal organism

Bean anthracnose is known to be caused by the fungous parasite *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav., one of the Melanconiales of the classification of Lindau (1900) as used in Engler and Prantl's *Die natürliche Pflanzenfamilien.* The fungus does not differ appreciably in its morphological characters from a number of other species belonging to this genus, nor, except for the presence of setae, from the genus Gloeosporium. Not only are species of the genus Colletotrichum and of the related genus Gloeosporium similar in appearance, but also the diseases produced by species of the two genera are similar in character.

Saccardo (1878), in his description of the fungus, gave it the name *Gloeosporium lindemuthianum* Sacc. & Magn. after Lindemuth, who discovered the fungus in 1875. Scribner (1888:364) described the presence of setae in the acervuli which were present on most specimens sent him from several States, and thought it probable that the generic name of the fungus should be changed to *Colletotrichum.* The following year Briosi and Cavara (1889) named the fungus *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav., and distributed it as such. The same year Scribner (1889), again finding setae in the acervuli, placed the fungus in the genus Colletotrichum and published it as *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scribner. The name given by Briosi and Cavara stands, although it is evident from much subsequent cultural work that the presence or absence of setae is not a character of sufficient stability to serve as a basis for the determination of generic position. Krüger (1913:294–303) was able to develop setose and non-setose cultures of this fungus at will, finding that a number of factors, such as age of the culture, and nature and moisture content of the substratum, were controlling agencies. He, however, designates the conidial form as *Gloeosporium* (subgenus *Colletotrichum*) *lindemuthianum* Sacc. & Magn.

Halsted (1893d), after making cross-inoculations on bean and watermelon, decided that the anthracnose fungi in these two hosts were the same, and used the name *Colletotrichum lagernarium* (Pass.) Ellis & Halsted, including in the synonymy the names that had been applied to the organism causing melon anthracnose and bean anthracnose, respectively. Shear and Wood (1913:46) found perithecia producing asci and ascospores in cultures made from an anthracnose spot on a bean pod, and they refer their fungus to *Glomerella lindemuthianum* Shear n. comb. Krüger (1913:311), although he did not find perithecia, designates the fungus as *Glomerella lindemuthianum* Shear n. comb. Edgerton (1915:255–256) questions whether any perithecial stage of this fungus has ever been seen, and suggests that Shear and Wood might have been working with one of the saprophytic forms occurring commonly on bean pods. He believes the fungus should be considered as a species of Colletotrichum until further evidence supports a different view.

At various times other names have appeared in the synonymy of this fungus. Richon (1889) gives the name *Glaeosporium phaseoli* n. sp. to a fungus which he found on the pods and stems of beans at St. Amand, France. His meager description fits this fungus very well, and it is not improbable that he had before him the fungus causing bean anthracnose. Saccardo (1892) lists *Gloeosporium phaseoli* Rich. and gives Richon's description. Allescher (1903) also lists Richon's fungus and gives his description, but says that it is an uncertain species perhaps identical with *G. lindemuthianum* Sacc. & Magn. or with a Colletotrichum.

Allescher (1903) designates as *Gloeosporium lindemuthianum* Sacc. & Magn. forma *foliicolum* Allesch. the fungus he found on the upper surface of subcircular, brownish spots on leaves of *Phaseolus vulgaris* var. *nanus.* An examination made of type specimen No. 380 Allescher & Schnabl (1894), in the herbarium of the Pathological Collection of the United States Department of Agriculture, showed spots somewhat larger and more nearly circular than is usual for anthracnose but not sufficiently distinct to warrant the placing of the fungus as a form of the species. The spores are like those of *Colletotrichum lindemuthianum.* Allescher says the form *foliicolum* is similar to the type except in the appearance of the spots as described and in the absence of setae.

Desmazières (1843a) describes as *Septoria leguminum* Nob. a fungus on the pods of Phaseolus. He ascribes to variety "*b*" of the same species a form on Pisum. Saccardo (1878) says he is not able to decide whether or not *Septoria leguminum* Dsmz. should stand, as he cannot distinguish the young condition from *Gloeosporium lindemuthianum,* but the description varies widely from Septoria and approaches more nearly that of *G. fructigenum* Berk. Berkeley (1881), speaking of dried specimens distributed by Desmazières under the name *Septoria leguminum,* says the species shown is not a good representative of the genus Septoria, but belongs rather to Gloeosporium and has been figured by Saccardo in his *Fungi Italici* as *Gloeosporium lindemuthianum.* Cooke (1881), on the basis of Berkeley's observations, cites *Septoria leguminum* Desm. as synonymous with *Gloeosporium lindemuthianum* Sacc. Kirchner (1906: 137) ascribes to it the small, dry, sharply differentiated brown spots on beans, on which appear later very small black pimples.

Specimens of bean pods distributed by Desmazières (1843 b) as *Septoria leguminum,* deposited in the cryptogamic herbarium of the New

York Botanical Garden, show the presence of the fructifications of two fungi on one of the two pieces of pods in the packet. One of these, occurring in characteristic young anthracnose spots, has the typical spores of *Colletotrichum lindemuthianum* except that the average width, 5.75 μ, is a trifle greater. There can be no doubt that it is the bean anthracnose fungus. The other fungus appears on the specimen as numerous small black pycnidia and these contain typical Septoria spores. The other specimen shows only the Septoria-like fungus. Desmazière's description of the fungus given in *Annales des Sciences Naturelles* (1843 a), and also accompanying the specimen, is of the Septoria, but his description of the disease applies, at least in part, to the condition produced by the Colletotrichum. This probably led to the mistake of ascribing to *Septoria leguminum* Desm. the anthracnose present on pods of *Phaseolus vulgaris* distributed by Von Thümen (1882) as *S. leguminum* and by Roumeguère (1884) as *S. leguminum* Desm. var. *Phaseolarum.* Saccardo (1896) describes a fungus, which he names *Gloeosporium socium* Sacc., as occurring on the leaves of living bean plants (*Phaseolus vulgaris*). But from his description it seems improbable that he has the bean anthracnose fungus. D. Saccardo (1904) labeled as *Colletotrichum lindemuthianum* (Sacc. et Magn.) Cavara f. *brachysporum* specimens of a fungus on bean pod, *Phaseolus vulgaris*, the conidia and basidia of which were recorded as considerably shorter than the normal average. An examination of type specimens in the herbarium of the New York Botanical Garden shows small but typical anthracnose spots, from which, however, no spores were obtained.

Heald and Wolf (1911) have ascribed to a new species, *Colletotrichum caulicolum*, a destructive canker which they found on the stem of Kentucky Wonder beans in Texas. They say that this fungus differs from *C. lindemuthianum* in that the setae are produced abundantly in the host and that the spores are larger and are falcate. Their description of the disease and of the fungus is similar in most respects to that of bean anthracnose and its pathogene, but it is not possible from the description to decide that the fungi are the same. Cooke and Harkness (1880) report *Gloeosporium leguminis* C. & HK. on legumes in California, and later (1884) on pods of Robinia at Sacramento, California. Cooke (1880) describes *Discella leguminum* Cooke on the pods of Prosopis found in Texas. Comes (1891) lists *Discella leguminum* Cooke and *Gloeosporium leguminis* C. &

Hark. as synonyms of *Gloeosporium (Colletotrichum) Lindemuthianum* Sacc. & Magn. An examination of type specimens of these two fungi in the possession of Dr. C. L. Shear, of the United States Department of Agriculture, leads to the conclusion that they are not at all related to the fungus here considered.

The synonymy would, then, appear as follows:

Gloeosporium Lindemuthianum Sacc. & Magn. Michelia 1:129. 1878.

Septoria leguminum Desm. *In* Von Thümen, Mycotheca universalis, no. 2096. 1882.

Septoria Leguminum Desm. var. *Phaseolarum*. *In* Roumeguère, Fungi gallici exsiccati, no. 2791. 1884.

Colletotrichum lindemuthianum (Sacc. & Magn.) Bri. & Cav. I funghi parassiti delle piante coltivate od utili, no. 50. 1889.

Colletotrichum lindemuthianum (Sacc. & Magn.) Scribner. Orchard and garden 11:193–194. 1889.

? *Glaeosporium phaseoli* Ch. Rich. Cat. raison. champ. Départ. Marne, p. 401. 1889.

Colletotrichum lagernarium (Pass.) Ellis & Halsted. Bul. Torrey Bot. Club 20:246–250. 1893.

Gloeosporium Lindemuthianum Sacc. & Magn. forma *foliicolum* Allesch. *In* Allescher and Schnabl, Fungi bavarici exsiccati, no. 380. 1894.

Colletotrichum Lindemuthianum (Sacc. et Magn.) Cavara f. *brachysporum*. Mycotheca italici, no. 1364. 1904.

Glomerella lindemuthianum Shear n. comb. *In* Krüger, K. biol. Anst. Land- u. Forstw., Arb. 9:311. 1913.

Morphology and physiology

Spores

Whether the fungus is grown on its host or in culture media, the spores are produced abundantly on the surface in acervuli masses of a grenadine pink color. Frank (1883 b:513) mentions the presence of a slimy substance with the spores in the acervulus, and this has since been noted by other workers as a gelatinous substance in which the spores are embedded. If such a mass of spores is placed in water, they separate and become suspended in the water so that they are readily disseminated by its dispersal. If the acervulus remains dry, it hardens and shrinks down to small gray or brown granulations, and the spores are seldom set free under such circumstances.

The summer spores (conidia) are oval or oblong, cylindrical, one-celled bodies of somewhat variable size (Plate III, 1). They are straight or slightly curved, with the ends rounded or at one end somewhat pointed. The dimensions, as averaged from a large number of spore measurements, are 15 by 5 μ. The largest spore encountered measured 22 by 5.33 μ and the smallest 13 by 4.44 μ. The content of young spores is homogeneous and granular. Often there is a clear body resembling a vacuole near the center, which Edgerton (1910:20) describes as a nucleus but which earlier workers have regarded merely as a vacuole. Edgerton says that as the spore ages it becomes vacuolate, and finally the contents collect in small masses. The spores are no longer viable when this last stage is reached.

Spore germination

In making germination tests, spores from young acervuli produced in bean-pod cultures were transferred to a drop of the culture medium placed on a flamed slide. The slides were transferred to sterile moist chambers or petri dishes kept moist with wet blotting-paper, in which they rested on glass supports to hold them up from the bottom. The temperature was maintained at about 20° C.

Germination usually takes place by the protrusion of a germ tube from the side of the spore near one end and at an angle to its long axis. On nutrient media some spores have germinated within eight or nine hours (Plate III, 2). Bean agar gives the quickest and largest percentage of germination, although nutrient beef agar, potato agar, and beef bouillon give nearly similar results. Soon after the protrusion of the first tube, a second may be sent out from a similar point at the other end. As many as four germ tubes have been observed coming from a single spore, but rarely are there more than two. Dey (1919:307) observed in a few cases more than two germ tubes developing from a spore. The germ tubes form branches which radiate from it in all directions (Plate III, 4).

Germination takes place more slowly in water than in agar. A few spores were observed to have germinated in water after eighteen hours (Plate III, 10). In certain rain-water cultures about one-half of the spores germinated in twenty-four hours, but this was a greater proportion than the average. In distilled water, germination proceeded somewhat more slowly. The majority of the spores in these water cultures became uniseptate during germination, but septation was not observed to occur so commonly in spores germinating in nutrient solutions. Often there is a constriction of the spore at the septum. In some cases the spores themselves increased somewhat in size before or during germination, but this was not general and when spores were placed in nutrient solutions it did not occur to any extent. Atkinson (1895) says that septation occurs unless the spore is very short, and that the spore becomes constricted at the point of septation soon after germination. He observed an increase in the size of the spores after germination until they became several times larger than at first, and states that even with spores which fail at first to germinate a similar increase in size takes place, and they become uniseptate, strongly constricted at the septum, and richly charged with highly refringent granules. Edgerton (1910: 19) states that the spores swell but slightly or not at all in germinating in nutrient media if they are scattering, but if they are abundant and close together in the culture medium they swell to two or three times normal size in some cases. During the process of enlargement a septum is formed, and with further swelling a constriction of the spore appears at this point. Edgerton finds that "when there are more than 12 or 15 spores to the cubic millimeter of medium, the spores become swollen, germinate slowly and but poorly. When the number is less than this, they germinate with but little, if any, swelling" (page 20 of reference cited). He thinks this may be due to an enzyme prohibiting growth, given off by the spore during germination. Dey (1919) observed that if the drop of water used in the culture had a convex surface, the spores lying in the middle hardly germinated while most of those at the border did. He thinks this difference is due to a difference in the oxygen supply.

The germination of the spore in water consists, in most cases, of the production of an appressorium at one or both ends of the conidium (Plate III). This is a subglobose body flattened on one side, having dark-colored, thick walls and containing dense granular protoplasm. It often appears attached directly to the conidium or is produced at the end of a short germ tube, although it is not uncommon to find an appressorium at the end of a long, narrow hypha. In the ordinary process of germination in nutrient media, the spore contents become coarsely granular and the vacuole increases greatly in size or there may be several vacuoles. In rain-water cultures, the spore contents during germination seem to pass into the appressorium, in which the protoplasm appears to be very dense.

The appressoria in most cases are closely appressed to the slide. A few have been noticed, however, which were apparently free, as they were observed to move when the slide was jarred. In the course of twenty-four hours some of these appressoria themselves send out a slender germ tube, at the end of which in most cases another appressorium is formed. After nine days about 50 per cent of the spores in this experiment had germinated but had not proceeded much beyond the production of appressoria. A number of spores had the appearance of being empty, and other peculiarities were noticed to which no importance could be attached.

Frank (1883 b: 513–515) describes the germination of the spore and the formation of appressoria, he being the first to apply the name to these bodies. These appressoria, Frank says, come to have a thick, irregular, dark purple membrane. They are produced abundantly when spores are germinated on the surface of bean or cucumber tissue, but seldom occur when germination takes place in water on a glass plate. Instead, long, thin, germ tubes are formed, at the end of which a small appressorium may appear, or the spore may produce another spore or sporidium attached directly to the spore or to a short tube. Frank says the appressoria do not germinate unless they are in contact with the host. He thinks their production is stimulated by the quality of the substratum, and suggests that their function is to prepare the fungus for penetration into its host plant.

Appressoria are produced by other Gloeosporia of the Glomerella type, and their significance has been discussed by other writers. Halsted (1893 a: 305) suggests that they may be formed as a protective body to tide the germinating spore over unfavorable periods. He says that, while the appressorium is often a single cell, it may become an aggregation of thick-walled cells like a sclerotium. He thinks the appressoria may be of taxonomic value. Stoneman (1898: 113) does not find them to occur as a constant character, but thinks they may be forced in certain species by lack of nourishment. Hasselbring (1906: 142), from studies of *Gloeosporium fructigenum* Berk., believes them to be adhesion organs during early stages of infection, and thinks they are formed as a result of contact stimuli of the germ tube but lose this power of reacting in nutrient media. He calls attention to the presence of a germ pore in the flattened side, through which the germ tube protrudes during germination. He finds the appressoria much more resistant to desiccation than the spores. Edgerton (1910: 21) finds appressoria produced in nutrient media, often not in contact with the glass or any solid substance; and in this species, *Colletotrichum lindemuthianum*, the "germ pore" is often lacking.

Gardner (1918:21), in his spore-germination studies of *Colletotrichum lagenarium*, concludes that a rather abundant supply of oxygen and a contact stimulus is necessary for appressorium formation, and that the presence of food material does not seem to inhibit such formation. Dey (1919), in his studies of *C. lindemuthianum*, states that appressoria are formed whenever the germ tube comes into contact with a hard foreign substance which acts as a stimulus for their formation. He found no germ pore in the appressoria observed, but noted that the germ tube arose from the side in contact with the glass. He observed the appressorium to be sheathed in a mucilaginous coat, which aids it in becoming attached to a foreign substance so firmly that even a jet of water fails to dislodge it.

In all the cultures made in fresh nutrient media, the germination was rapid, the germ tubes were as large, or nearly so, as the spores, few or no appressoria were formed, and the subsequent development of the mycelium was luxuriant; while in water cultures, the germination was slow, the germ tubes were much smaller in diameter, the production of appressoria was abundant, and subsequent development of mycelium was scanty or there was none at all. It is true that appressoria were commonly produced in nutrient media that was becoming somewhat dry and hard, as occurred soon when only a little was poured into a petri dish. When a nutrient medium is poured over a water culture, as suggested by Hasselbring (1906), the appressoria germinate within a few hours and produce a rich mycelial growth.

Mycelium and spore production in culture

Under favorable conditions for growth, the germ tube branches to form hyphae, and these in turn, by further growth and branching, develop within forty-eight hours into a mycelial mat often one millimeter in diameter. In three or four days conidia are produced at the ends of the hyphal threads. In several agar cultures in this experiment they were produced abundantly on short threads within twenty-four hours. They are formed by the constriction of the wall near the extremity of the hypha. A spore thus formed is pushed aside and the hypha elongates to about the same point, when constriction again occurs. In this way several spores are formed from a single branch, and can be seen lying side by side or piled in a heap at the end of the branch (Plate III, 5). Atkinson (1895:309) observed conidia being produced directly on the spore, and in the writer's cultures this condition was frequently met. Atkinson found that the ends of the hyphal threads have a dichotomous appearance, due to a branch just behind the growing end overtaking the primary thread, and that a plumose tuft is produced by this dichotomous branching taking place successively in the same thread.

The mycelium, which at first is white, will become dark-colored after a growth of several days in a starch-containing medium. This is especially noticeable with the growth in potato agar and bean agar and on sterilized bean pods. A culture four or five days old shows a dark center with pure white mycelial growth at the margin and aërial mycelium developing over the surface. As growth continues, dark-colored knots of mycelial threads appear scattered from the center outward, and above these darker parts of the culture, flesh-colored masses of spores (acervuli) begin to be produced if the culture is in good condition. Dey (1919) finds that in cornmeal agar cultures these spores are surrounded by small dark spines. On sterilized bean pods inoculated with spores kept at a temperature of about 22° C., there is a rapid growth of mycelium which in two days begins to darken and after three days produces spores in acervuli. In six days the pod is covered with a pale salmon or pale flesh-colored mass of spores. Cultures kept for a long time or at unfavorably high temperatures lose their power to develop spores in this way when transferred to fresh media. Commonly they develop only a slow growth of white mycelium. Spores will usually be produced if the mycelium turns dark.

Viala and Pacottet (1905), from a study of *Colletotrichum lindemuthianum* in culture, report the production of spermagonia, pycnidia, cysts, yeasts, and sclerotia, as well as conidiophores, similar in most respects to those produced by *Manginia ampelina*, another fungus under their observation. Shear and Wood (1913:64) say yeasts have never occurred in their cultures of Gloeosporia except where they were evidently contaminations, and they have never observed the production of spermagonia or pycnidia by these fungi. No other writers in speaking of *C. lindemuthianum* or related forms have mentioned the production of bodies of these kinds, except that Edgerton (1910:10) reports having once found pycnidia-like bodies in the tissue of the bean seed.

Temperature is an important factor in the development of the fungus in connection with its host or in culture media. Edgerton (1910:29) found that the fungus died in culture at Baton Rouge, Louisiana, during the summer, even when frequently transferred to fresh media, and later (1915) showed that the temperature requirements for this fungus are lower than for other anthracnose fungi, the optimum being about 22° to 23° C. though there is but little difference in growth between 19° and 26°. Edgerton gives the maximum as about 30° or 31° C. The growth at optimum temperatures is between 3 and 4 millimeters a day. Muncie (1917:9–11), after subjecting test-tube cultures of old mycelium to a water bath at 65° C. for ten minutes, found that good growth could yet be obtained but that the thermal death point of young mycelium lies between 50° and 52.5° C. and that of spores between 45° and 48° C.

The writer endeavored to determine the minimum, optimum, and maximum temperatures for growth and production of spores. Potato agar plate cultures of two physiologically different strains of *Colletotrichum lindemuthianum* were kept for a few days at 25° C., after which blocks of agar 3 millimeters in diameter of each strain were transferred to plates of potato agar and corn agar and to tubes of sterilized bean pods. The temperatures (centigrade) at which the cultures were placed were, respectively, 0°, 4°, 10°, 16°, 22°, 28°, 34°, 40°, 43°. Two cultures of each strain in potato agar and two of each in corn agar were placed at each of these temperatures on April 8, 1914. The growth made by them is shown graphically in figure 12.

It is seen that the average growth during the first six days was less than on the eight succeeding days at the temperatures 4° and 10° C., but was greater at temperatures above 28° C. Growth was probably checked during the first day or two at the lower temperatures by an abrupt transfer from a warm room, but was resumed to some extent later. While the growth was no doubt checked by the transfer to the higher temperatures, and ceased altogether after a few days at 34° and above, yet some growth was possible during the first day or two because the temperatures then ranged between room temperature, at which the cultures were started, and the higher temperatures. The figures giving the average growth on the eight days following the first six represent more nearly accurately the actual growth that occurs at these temperatures under these conditions. Considering, then, the curve representing such growth, it appears that the minimum temperature for growth lies at 0°, the optimum at 22°, and the maximum at 34°. As a matter of fact, the minimum lies somewhere between 0° and 4° C., the optimum near 22°, and the maximum between 34° and a few degrees lower. This corresponds very closely to the observations of Edgerton (1915:254). The corn agar permitted a little better growth than the potato agar.

The growth at 4° C. was white, with considerable aërial mycelium in most cultures. After four weeks a growth of from 25 to 35 millimeters was attained, and an olivaceous color, with spore production, was noticed in a few agar and bean-pod cultures. Spores were produced in abundance on bean pods at 10°, 16°, and 22° in six days, and but sparingly at 28°. As already stated (page 121), bean pod cultures kept at 22° C. have been found to give a rapid and abundant production of spores when the fungus was in a spore-producing condition, in hundreds of cultures made by the writer.

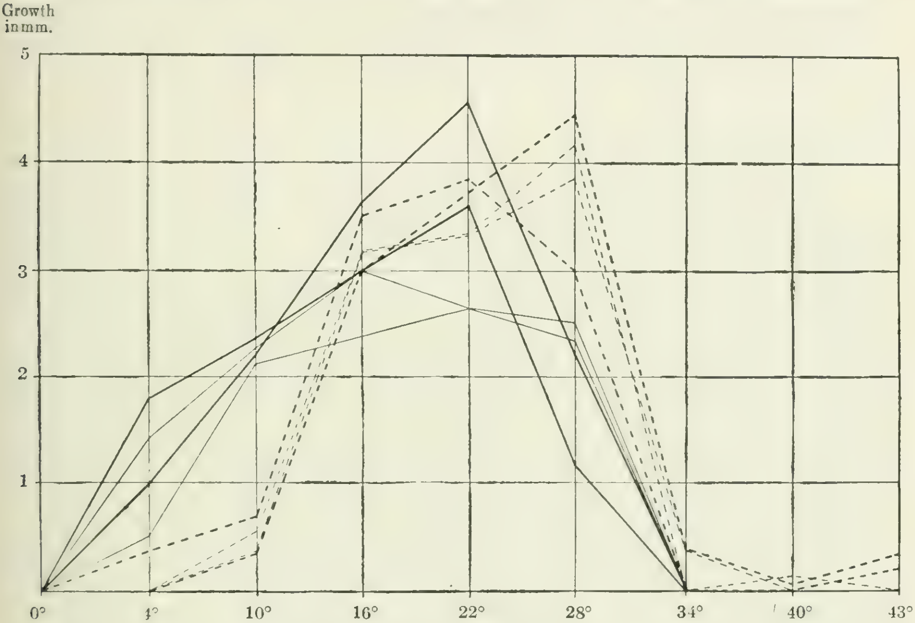

FIG. 12. CURVE OF GROWTH OF TWO STRAINS, A AND B, OF COLLETOTRICHUM LINDEMUTHIANUM AT VARYING TEMPERATURES

The light curves represent growth in potato agar, the heavy curves growth in corn agar. The dotted lines represent average growth per day during the first six days; the straight lines, average growth per day during the following eight days. The temperature is expressed in degrees centigrade

The relation of growth of this fungus to acid and alkaline media has not been studied by the writer, though he has observed that not as good growth is secured when the fungus is cultured in a potato-agar medium made acid by the addition of one drop of 50-per-cent lactic acid to 10 cubic centimeters of medium, as when such acid is not added. Edgerton (1910:28) records a fair growth of *Colletotrichum lindemuthianum* at −16 Fuller's scale, excellent growth at +5, and only slight growth at +20. *Glomerella gossypii* (South.) Edg. grew better than did *Colletotrichum lindemuthianum* in the more acid media.

The ascogenous stage

The ascogenous stage of many anthracnoses has already been found in nature or produced in culture. Klebahn (1905) has shown that the Gloeosporium causing sycamore anthracnose is a Gnomonia. He also (1907:65) found the perfect stage of *Gloeosporium ribes* (Lib.) M. & D., causing an anthracnose of currant leaves, to be a Pseudopeziza. Burkholder (1917:164) discovered the perfect stage of *Gloeosporium venetum* Speg. on raspberries to be a Plectodiscella, which he named *P. veneta* Burkholder. Genera representing perfect stages of other Gloeosporia that have been reported (Stevens, 1913) are Glomerella, Gnomoniella, Gnomonia, Trochila, Physalospora, and Calospora. Most of the anthracnose fungi parasitic on fruits and herbaceous plants, including the one on bean, belong to what is known as the Glomerella type, that is, their perfect stage when found is of the type of the genus Glomerella. Even in their imperfect stage they differ somewhat from the other forms. Edgerton (1908:384) has pointed out the following important differences, among others of minor importance:

Glomerella type	Gnomonia and Pseudopeziza types
Spores oozing out as a pink mass	Spore mass white, cream-colored, or yellow
Appressoria produced in certain cultures under certain conditions	No appressoria observed
Mycelium in culture varying but little in diameter	Mycelium in culture varying in diameter (at least in Gnomonia type)

Stoneman (1898:99–112) was the first to discover the ascogenous stage of a Gloeosporium of the Glomerella type, and she described five species occurring in nature or in culture. These were placed in a new genus of the Gnomoniaceae which Stoneman named Gnomoniopsis. Since then the perfect stages of at least thirty-two other Gloeosporia or Colletotricha have been found in nature or produced in culture. Von Schrenk and Spaulding (1903), because of a prior use of the name Gnomoniopsis, substituted for it the name Glomerella.

Shear and Wood (1907, a and b, and 1909) have produced in culture the perfect stage of anthracnose fungi from a large number of hosts, and among them what they believed to be the ascigerous perithecia of *Colletotrichum lindemuthianum.* They say that light, temperature, and moisture are not factors of much importance in producing this stage. They hold that once a race, a strain, or a generation is obtained that will produce the perithecia, these may be produced in various media and under diverse conditions. Moreover, they state that if a given strain will not produce perithecia in sterilized cornmeal at 75° to 85° F., it is useless to try longer to obtain them from that strain. In a later publication (1913:14), after having studied forms on forty-five different hosts and having obtained the ascogenous stage from thirty-six hosts, they refer forms from thirty-nine hosts to *Glomerella cingulata* (Stonem.) S. et v. S., the one from cotton to *Glomerella gossypii* Edg., the one from bean to *Glomerella lindemuthianum Shear*, those from watermelon, cucumber, and squash to *Gloeosporium lagenarium* (Pass.) Sacc. & Roum., and the one from banana to *Gloeosporium musarum* Cke. & Mass.

In the same publication (1913:46–47) Shear and Wood record the finding of perithecia producing asci and ascospores in flasks of cornmeal from cultures made from an anthracnose spot on a bean pod. While the perithecia were abundant, conidia were scarce or wanting. Numerous subsequent plate cultures made from crushed perithecia and asci and from single ascospores produced the usual growth of mycelium and abundance of perithecia with mature asci, but no conidia were seen. Shear and Wood believe this form to be an extreme variation of a condition they have noticed in respect to cultures from other hosts, in that cultures from ascospores produce fewer conidia than do cultures made from conidia.

Edgerton (1915:255) thinks it doubtful that any perithecial stage of *Colletotrichum lindemuthianum* has ever been seen, and that Shear and Wood were not working with a culture of the true bean-anthracnose fungus but rather with one of the saprophytic forms which Edgerton finds occurring commonly on old parts of bean and other plants. These forms produce perithecia abundantly, but no conidia. In hundreds of cultures of *C. lindemuthianum* examined by him, no perithecia have been found, and he thinks that until more satisfactory proof is offered of the presence of perithecia it is better to consider the bean anthracnose fungus as lacking an ascogenous stage.

Relation of fungus to host

Penetration of the host

Frank (1883b:515) described in detail the germination of the spore and the penetration of the host. He says that the spore, on germinating, produces a germ tube, which on coming into contact with the epidermis of the host forms an appressorium closely pressed against the surface of the epidermis. This either remains unchanged, or after a time becomes thinner and colorless on one side, and from this spot a germ tube appears. The germ tube penetrates directly through the cuticle into the epidermal cell, and either fills this cell with branching hyphae or quickly penetrates the adjoining epidermal cells or those beneath. Voglino (1892), from inoculations of parts of green bean pods, observed the germination of the spore, the quick formation of large numbers of appressoria, and the production of mycelial filament which penetrated into the interior of the epidermal cells. Dey (1919) has carefully studied and figured this penetration from stained sections made from material embedded in paraffin. He finds that a peg-like infection hypha grows out from the surface of the appressorium in contact with the host, which mechanically ruptures the cuticular layer and then brings about a swelling and disintegration of the subcuticular layers — probably by enzymic action. The infection hypha was never observed earlier than forty-eight hours after inoculation. The rupture of the cuticle is the result of the pressure exerted by the development of the infection hypha. This hypha is very fine at first, but reaches a normal size while growing in the cellulose layers below the cuticle. Here, or farther on in the cell, it produces a small vesicle from which one or more branches arise and extend into the host tissue. As the invading hypha enters the cell, the protoplasmic contents collect around it, and later a collapse of the cell takes place. The writer has never observed this penetration. It seems that this is the really critical time in the development of the fungus, for it must overcome the protoplasm of the host cell before it can continue its development. Varieties of beans immune to certain strains of *Colletotrichum lindemuthianum* probably owe this immunity to the inability of the infection hypha of the fungus to overcome the resistance offered it by the protoplasm of the host cell. The writer (Barrus, 1918:594) has shown that some such varieties, on inoculation with such a strain, have shown on their pods and stems a slight but distinct specking, as though the fungus had entered the tissue and killed a few cells but was unable to proceed farther. This specking did not appear on the pods of plants not inoculated.

Once the fungus has established itself within the host cell, the hyphae extend horizontally and diagonally into other cells, penetrating the cell walls of the host as they come into contact with them (Plate II). In some cases the hyphae enlarge when in contact with the cell wall, and a small hole is formed through which projects a slender tube that at once enlarges after passing through. As many as twelve hyphae have been observed penetrating a single cell (Plate II, 4). Growth within the host is rapid, particularly if the tissue is young and tender. The protoplasm of the host cell is killed and turns brown soon after being attacked. The walls collapse, and after from four and one-half to seven days a lesion may be observed on the surface.

There seems to be a great difference of opinion as to the length of the period of incubation. Frank (1883a:33) noticed the beginning of a browning of the epidermis at separate points on the pods twenty-four hours after inoculation was made. This browning had extended by the following day, and five days after inoculation Frank obtained the first spores in the new lesion. Scribner (1888) states that the cells become discolored almost at once after the hyphae enter. Halsted (1892:284) found a spot on an otherwise healthy pod thirty-six hours after inoculating it in the laboratory under the most favorable conditions. Pammel and King (1909) report having obtained an anthracnose spot thirty-six hours after inoculation. Dey (1919) claims that early specking appears on a pod when tap water is substituted for infection drops, and thinks it due to osmotic disturbances. Edgerton (1910:14) finds from inoculations made on plants in the greenhouse and in the field that the period of incubation ranges from four and one-half to nine days, depending on the weather and on temperature conditions. The writer's numerous observations are in accord with this as regards the production of typical anthracnose lesions. Temperature appears to have some influence on the length of the incubation period; also, the period varies considerably with the different varieties of beans under the same conditions. Schaffnit (1920) finds that the incubation period, and also the period between infection and spore formation, differ with different varieties.

Reproduction of the fungus within the host

At about the time when the cell walls collapse to form a canker, or even earlier in many cases, the mycelium at certain points develops abundantly in the epidermal cells and in those just beneath, forming a stroma of closely crowded, many-septate hyphae. The host cells at these places are so flattened that it is difficult to observe them (Plate II, 2) From this pseudoparenchymatous tissue arise short, more or less erect, conidiophores, from 15 to 20 μ long and from 4 to 5 μ broad, at the apex of each of which is borne a small conidium rounded at the top and drawn out to a point at its attached end. This conidium enlarges somewhat before becoming detached. The conidia are formed by the constriction of the upper end of the conidiophores, as described in connection with germination in culture media. Spore after spore is produced by the conidiophore; the cuticle, which has been pushed up to form black pimples in the canker as the conidiophores elongate, is soon ruptured, and the spores emerge in a gelatinous pink mass (Plate I, 1) Frank (1883b: 513) describes the formation of the stroma and the production of spores enveloped in slime, the rupture of the cuticle, and the behavior of the spores thereafter.

These first acervuli formed at the center are followed by the production of others as the lesion enlarges. In a single lesion there may be fifty or more acervuli, in which thousands of spores are produced. Edgerton (1910: 12) estimated that on a badly spotted pod, from 110,000,000 to 115,000,000 spores had been produced. After washing these off and placing the pods in a moist chamber for twenty-four hours, he found that about 50.000,000 more spores were produced, and he concluded that a total of from 500,000,000 to 1,000,000,000 spores may develop on some pods. If the weather remains moist and cool, the conidiophores will continue to produce conidia; but during dry weather the spore masses harden down over the acervulus and spore formation ceases.

As the acervuli become older, long-pointed, brown, septate hyphae (setae) are produced here and there among the conidiophores and often in a ring near the margin. These range in length from 30 to 90 μ. Some taper evenly to a point from a base 8 to 10 μ wide; others have a bulbous base, which Beach (1892:319) reports as being often many-celled; many do not taper evenly but are swollen near the septa. They vary in number from a few to as many as twenty in an acervulus (Plate I, 2). Black stromata made up of dark-colored hyphae are produced on the surface of dead areas of leaves spotted by anthracnose and on the surface of dry pods, and these invariably support setae. Setae are abundantly formed also in old agar and bean-pod cultures. Frank (1883 b:517) found them in lesions on bean pods, and describes them as sterile, brown, hair-like appendages of the fungus.

The fungus in the seed

The fungus passes the winter in the seed as mycelium lying within the cells in a more or less dormant condition. It may be confined to the cells of the seed coat, but in badly diseased seeds it occurs in the cells of the cotyledon. It exists there as vacuolate, closely septate, hyphae, from 3 to 5 μ in diameter, which are wound about in the cells or extend diagonally across into other cells. It may occasionally be found as a cobwebby growth between the seed coat and the cotyledon, or even between the two cotyledons within the seed. Frank (1883 b:520) found spores produced in lesions of the seed, Scribner (1888) says that spores and basidia may be found between testa and embryo, and Halsted (1892:285) found them borne in the cavity between the cotyledons of the diseased dry seed. Halsted called attention to the rapid growth of the fungus and to the production of spores on the diseased seed when it is placed under a bell jar. Edgerton (1910:10) notes that acervuli are often present on the surface of the spots and between the cotyledons, and finds peculiar closed pycnidia-like bodies buried some distance beneath the surface of the bean.

Gain (1898) has shown that the density of attacked seeds may be lowered as much as 4.7 per cent. Muncie (1917:37) states that badly affected seed will remain on the surface of a solution of sodium nitrate of given concentration, while slightly affected and clean seed will sink in it.

The viability of the seed also is affected according as the mycelium has or has not penetrated beyond the epidermis which protects the embryo. Gain (1898 and 1899) says that 10 per cent of the affected seed planted did not germinate, 8 per cent germinated but did not grow, and 36 per cent, although affected, were viable; while 46 per cent having only the integument but not the cotyledon affected produced healthy plants, as the fungus cannot produce fructifications unless it has penetrated into the cotyledon. Seed affected to one-twentieth of their volume are not viable. Gain says also that the disease can be quickly communicated from one seed to another by contact, or by inoculation with spores. Halsted (1892) states that only half of the diseased seed germinated, and those that did germinate produced plants of a sickly nature. Observations made by various other workers seem to prove that affected seed germinates poorly. The experience of the writer indicates that the percentage of germination of anthracnose-affected seeds is dependent mainly on the tissue affected and the area of the lesion. If the embryo or the tissues near it are affected, the seed usually will not germinate. When conditions are far from optimum for germination of bean seed, affected seeds often do not germinate, but neither will healthy seed germinate well under such conditions. The larger proportion of plants appearing from healthy seed than from affected seed under unfavorable conditions for germination is due to the attack of the new tissues by the anthracnose fungus and sometimes by other fungi associated with it. Edgerton (1910:36) obtained a much lower percentage of germination from anthracnose-spotted seed planted in unsterilized soil than from that planted in soil that had been sterilized, due to rot organisms in the unsterilized soil which attacked such seed. He says, however, that in the latter case the great majority of plants appearing were destroyed by anthracnose, while in the former there was greater freedom from the disease, indicating to him, as did other trials and observations, that rot organisms of the soil, especially a certain Fusarium commonly associated with anthracnose spots on seed after planting, will in Louisiana greatly reduce the severity of the anthracnose.

It appears that when a diseased seed is placed in a fairly moist situation, the threads of the fungus, theretofore dormant, renew their activity, extending into healthy cells beyond and forming spore-bearing conidiophores at the surface of the lesion. These conidiophores appear first as small black pimples in the lesion, from which spores issue in small, pinkish, pasty masses. As already mentioned, if the acervulus remains dry these spores harden down to little gray or brown granulations, and the spores are seldom set free under such circumstances. Ordinarily the lesion becomes wet with soil moisture or later with rain or dew, and then the spores separate from one another and become suspended in the water. Sometimes the entire seed becomes enveloped by a moldy growth of the fungus and fails to germinate; but when a diseased seed is viable and soil conditions are favorable, spores may be produced in the diseased parts of the cotyledons by the time they have appeared above ground. If the soil is sufficiently moist as the seed is emerging from the ground, spores are set free and these are able to infect the young and tender stems.

Infection of the seedling from affected seed

The plumule, consisting of the young leaves, is during germination and for a short time thereafter in intimate contact with the cotyledons (fig. 13). The water containing the suspended spores, by capillarity and

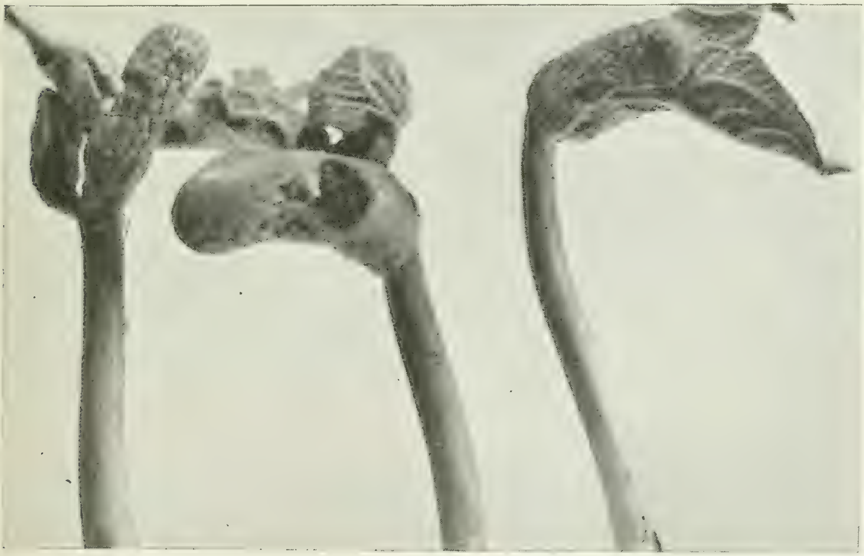

FIG. 13. SEEDLINGS PHOTOGRAPHED TO ILLUSTRATE MANNER OF INOCULATION OF JUVENILE LEAVES FROM SPORES PRODUCED ON COTYLEDON

Seven-eighths natural size

in other ways comes into contact with the now exposed under surface of these leaves. The stem may also become inoculated with spores that have been washed down from the cankers on the cotyledons, especially the base of the stem just below the surface of the soil, where moisture conditions are favorable for the germination of the spores. Frank (1883b:522) says that after affected cotyledons fall from the growing seedling, the fungus continues to grow in them and produces spores in great abundance which are washed by rain into the soil and bring about an infection of the plant in this way. Spattering of contaminated water and of soil also serves to inoculate the seedling; this is discussed more fully later.

Dipping seed in a suspension of spores just before planting will bring about an infection of the cotyledons and other parts of the seedlings, as has been shown by Edgerton (1910:41) and verified by the writer. Gain (1898) proved that the soil can become contaminated and serve as a source of inoculum.

Seeds planted in soil to which water containing spores of this fungus is applied will also produce infection. On August 22, 1910, thirty-six clean bean seeds were planted in twelve pots and watered with a suspension of spores of *Colletotrichum lindemuthianum*. As a check, six seeds were planted in two pots to which water without spores was applied. On September 6 thirty-one plants had appeared in the twelve pots, of which thirty, or 96.8 per cent, were infected. None of the six plants that appeared in the check pots were affected. On August 30, 1910, fifty clean seeds of the variety Golden Refugee were planted in a flat in clean soil, and water containing spores of *C. lindemuthianum* was poured over the surface until the soil was wet. On September 9 forty-nine plants had appeared, of which six showed anthracnose lesions. No later observations were made.

Longevity of fungus in soil

In order to find out whether spores washed to the soil would remain viable over winter, the following experiment was carried out. On November 27, 1916, twenty-five 8-inch pots were filled with loam and sterilized in the autoclave for three hours at twenty pounds pressure. On December 15, fifty test-tube cultures of *C. lindemuthianum* grown on sterilized bean pods were placed in 4 liters of tap water and the spores were washed off. The suspension was then filtered through a mat of absorbent cotton to eliminate fragments of plant tissue. Twenty-one pots of soil were inoculated by pouring over the surface of each about an equal portion of the suspension. There being some of the suspension left over from the first application, a double portion was applied to three pots. Four pots did not receive any spores, and these were labeled *Checks*. Several thicknesses of cheesecloth were tied over the top of each pot and the pots were placed outdoors. In the course of the winter and spring, the pots were taken into the greenhouse and planted with Davis White Wax, a susceptible variety, the seed used having come from healthy pods and having been carefully sorted. Twenty-five seeds were planted in each pot. After the beans were planted, unused sphagnum was placed over the surface to the depth of an inch to keep the surface of the soil in a moist condition. At the last trial, on June 8, four of the pots were inoculated again with a fresh suspension of spores as a control. The results are given in table 3. It was thought that the pots planted on

TABLE 3. RESULTS OBTAINED BY GROWING BEANS IN SOIL THAT HAD BEEN INOCULATED WITH A SUSPENSION OF SPORES OF COLLETOTRICHUM LINDEMUTHIANUM, AS COMPARED WITH THOSE GROWN IN UNINOCULATED SOIL

Date of planting	Date of examination	Type of inoculation	Number of plants appearing	Number of plants affected	Remarks
Dec. 15, 1916	Jan. 6, 1917	2 single	25	11	Small lesion at base of stem
			25	2	Ditto
Jan. 30, 1917	Feb. 25	1 double	21	17	Well-defined lesions on stem
		1 single	24	2	Ditto
		1 check	23	0	
April 13	May 7	2 double	25	0	
			24	0	
		3 single	24	0	
			25	0	
			25	0	
		1 check	24	0	
Pots replanted May 7	May 29	2 double	25	4	Well-defined lesions on stem. All 4 affected plants adjoining
			24	0	
		3 single	22	0	
			22	1	1 small lesion on stem
			24	0	
		1 check	24	0	

TABLE 3 (*continued*)

Date of planting	Date of examination	Type of inoculation	Number of plants appearing	Number of plants affected	Remarks
June 8	June 24	8 single	23	0	
			24	1	5 old lesions on stem
			25	0	
			23	0	
			22	3	1 with several old lesions. 2 with new lesions, apparently secondary infection
			25	2	2 with one new lesion, apparently secondary infection
			25	0	
			25	1	4 old lesions
		2 checks, not inoculated	25	0	
			25	0	
		4 recently inoculated	24	24	Stems badly affected
			22	22	Ditto
			22	19	Ditto
			24	17	Ditto

April 13 may not have given infected plants because the surface of the soil had been allowed to remain dry for some time through neglect. As no new spores had been produced to vitiate the experiment, these pots were replanted on May 7.

It would appear from this experiment that a few spores are able to live over winter in the soil outdoors and bring about infection the following spring. It is but fair to say, however, that the plants were not in any case screened from greenhouse insects that were present, and that other inoculation experiments with bean anthracnose were being conducted by other persons in another compartment of the same house and in adjoining houses. This may account for the few cases of disease occurring in the spring experiments. The infection is very light compared with that occurring on plants grown in freshly inoculated soil. Under practical bean-growing conditions the chances of infection from this source must be negligible.

An experiment similar to the one recorded above was tried the following winter, the same methods being employed. A greater effort was made, however, to keep the surface of the soil moist at all times, and also unsterilized but presumably uncontaminated soil, as well as sterilized soil, was used. On January 5, 1918, all the pots were well moistened with tap water, soon after which a suspension of spores was poured over the surface of the soil of those to be inoculated. Sterilized sphagnum was then placed over the soil to the depth of an inch, and covers were tied over the tops of the pots. After three days some of the pots were placed outdoors, being set in the soil so that the tops were level with the surface of the ground; others were left inside the violet house, where the temperature is kept cool. Check pots not inoculated were similarly prepared. At each planting made thereafter a control pot, made up of fresh uncontaminated soil, was inoculated in the same manner as were the pots inoculated earlier. Twenty-five clean beans of the variety Refugee Wax were planted in each pot except in the first planting, when the variety Extra Early Refugee was used. After planting, the pots were kept in the warm house. The results are given in table 4.

TABLE 4. RESULTS OBTAINED BY PLANTING BEANS AT VARIOUS TIMES IN SOIL INOCULATED WITH A SUSPENSION OF SPORES OF COLLETOTRICHUM LINDEMUTHIANUM, AS COMPARED WITH THOSE GROWN IN UNINOCULATED SOIL

Date of planting	Date of examination	Description of pots used	Number of plants appearing	Number of plants affected	Remarks
Jan. 5, 1918	Jan. 25, 1918	Check (not inoculated):			
		Soil sterilized	25	0	
		Inoculated:			
		Soil not sterilized	22	21	Varying from 2 excellent, to those with a slight lesion at base of stem
Jan. 17	Feb. 17	Check (not inoculated):			
		Pots left outside:			
		Soil sterilized	17	0	
		Inoculated:			
		Pots left outside:			
		Soil sterilized	21	1	Slight lesion on stem
		Soil not sterilized	7	0	
		Pots left inside:			
		Soil sterilized	23	2	Fair halfway up stem
		Soil not sterilized	17	5	One or more lesions on stem
Feb. 20	March 11	Check (not inoculated):			
		Pots left outside:			
		Soil sterilized	21	0	
		Soil not sterilized	13	0	
		Inoculated:			
		Pots left outside:			
		Soil sterilized	20	0	
		Soil not sterilized	15	0	
		Pots left inside:			
		Soil sterilized	20	0	
		Control (fresh soil used):			
		Soil inoculated 2-20-18	19	19	Lesions, many of them large, on stems

TABLE 4 (*continued*)

Date of planting	Date of examination	Description of pots used	Number of plants appearing	Number of plants affected	Remarks
April 3	April 19	Checks (not inoculated):			
		Pots left outside:			
		Soil sterilized.......	24	0	
		Soil sterilized.......	18	0	
		Inoculated:			
		Pots left outside:			
		Soil sterilized.......	22	0	
		Soil sterilized.......	19	0	
		Soil sterilized.......	19	0	
		Soil not sterilized....	25	0	
		Soil not sterilized....	18	0	
		Pots left inside:			
		Soil sterilized.......	24	0	
		Soil sterilized.......	19	0	
		Soil not sterilized....	13	0	
		Control (fresh soil used):			
		Soil inoculated 4–3–18..	16	13	Excellent on stems

It appears fairly evident that spores of *C. lindemuthianum* removed from the acervulus and remaining in the soil for a period of two weeks or more are able to bring about an infection of susceptible bean seedlings grown in such soil, but not after six and one-half weeks. Under field conditions, however, in addition to spores being washed into the soil, there are many bits of plant tissue, some of which would probably be infected tissue, remaining on the soil after the beans are harvested.

Frank (1883 b:521) tested the germination of spores which he found produced in the lesions of dry seed. He concludes that spores attached to the basidium remain viable throughout the winter, while those produced earlier and unattached are unable to survive. Edgerton (1910:25) has studied in a different way the question of the viability of these spores. He finds that the old spores on or within affected seeds are capable of germinating in nutrient media, and suggests the possibility of the infection of seedlings from healthy seeds by such spores, they having been transferred to the healthy seed by contact with affected ones. He finds also that good germination takes place in agar after the spores have remained dried for thirteen days in their mucilaginous matrix or washed free from it, and states that some germination took place after twenty-two days had elapsed.

Infection was also brought about, in an experiment, by placing recently affected vines or pods on or in the soil before the bean plants appeared. On August 8, 1910, vines of the current year badly affected with anthracnose were placed in a flat and covered to the depth of an inch with soil. On the surface of this soil, fifty hand-sorted Refugee Wax bean seeds were planted. These were covered lightly with soil. The soil came from a neighboring field that had not produced beans for years. The flat was then placed outdoors and watered once with well water. All the seeds produced plants, which on August 22 were not affected on any part above ground. On August 29 forty-two plants had appeared, of which thirty-five, or 83⅓ per cent, showed lesions on stem, on leaves, or on both.

On August 22 one hundred clean Refugee Wax seeds were planted in clean soil in a flat and covered lightly with soil. Over the surface was placed a quantity of immature pods badly spotted with anthracnose and producing spores. On September 6 ninety-eight plants had appeared, of which ninety-four, or 95.9 per cent, were infected.

Halsted (1896:286) has shown how beans planted in ground on which diseased plants were grown the year before, gave from four to six times as many spotted pods as did beans grown on new land; he found also (1896:288 and 1897:330) that mulching the soil with diseased pods from the preceding season caused an increase in the number of spotted pods over those in soil mulched with hay. This would lead to the belief that the fungus is capable of living over winter as spores in the soil or as spores or mycelium in the diseased pods.

Observations were made each year from 1910 to 1914 to determine whether soil which the preceding year had produced plants badly affected with anthracnose could serve as a source of inoculum to seedlings. Healthy seed planted in such soil gave in every instance seedlings that were free from anthracnose and remained so for a considerable time afterward.

Tests were made to determine whether the fungus can live over winter in old pods and vines. Badly spotted pods stored in paper sacks in an attic were found during the late winter and early spring to possess a thin black mycelial growth over their surface outside of the old lesions, and scattered thickly in places on this growth were small black bodies resembling pycnidia as viewed with a hand lens (fig. 14). A section through these bodies examined under the microscope showed them to be compact

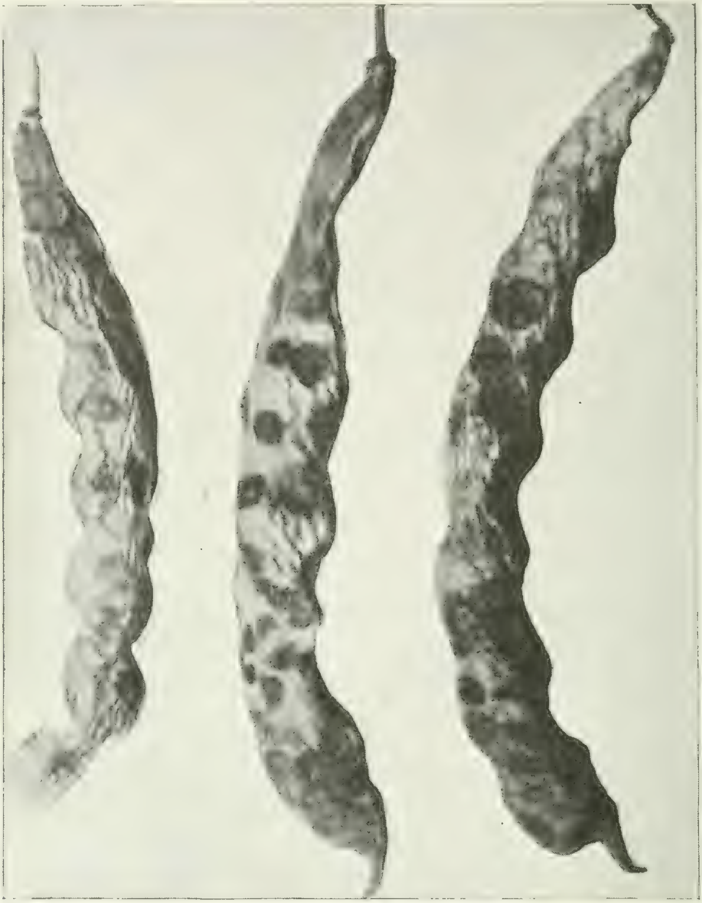

FIG. 14. SAPROPHYTIC DEVELOPMENT OF ANTHRACNOSE ON SURFACE OF DRY PODS

acervuli containing a large number of setae. The conidia, present in large numbers, germinated readily in a nutrient solution, and when healthy plants were inoculated with them anthracnose lesions appeared. The growth seems to be of a saprophytic nature, as the pods were dry when picked and at that time none of this growth was observed. Other isolations of *C. lindemuthianum* have been made from dry bean pods from time to time, even from pods more than a year old.

Trials were made also to determine whether old pods and vines affected by anthracnose may serve as a source of inoculum to seedlings. In the fall of 1911 a plot of ground where anthracnose had appeared to a severe extent on beans was selected for experiment. The diseased vines were left there, and diseased vines from other places were spread on the plot, all being plowed under the following spring. On June 13 a short row of each of five varieties was planted with seed from selected healthy pods. Adjoining these, one row of each of two varieties was planted with seed spotted with anthracnose. The seedlings were appearing on June 19. In several examinations made before June 25, the plants from the healthy seed showed no affection with anthracnose, while the young leaves of the plants from spotted seed were severely affected. Eight plants from healthy seed were found to be affected on July 1 as shown in table 5. This may have been due to secondary infection. The order in which the varieties appear in the table is the order in which they were planted in the plot.

TABLE 5. RESULTS OF AN EXPERIMENT TO DETERMINE WHETHER OLD PODS AND VINES AFFECTED WITH ANTHRACNOSE CAN SERVE AS A SOURCE OF INOCULUM TO SEEDLINGS
(Seed planted on June 13, 1912; data taken on July 1, 1912)

	Varieties planted with healthy seed					Varieties planted with seed spotted with anthracnose	
	Blue Pod Medium	Turtle Soup	California Cream	Navy Pea	Red Marrow	Navy Pea	Red Marrow
Number of plants appearing	306	492	346	1,070	1,473	198	262
Number affected with anthracnose	0	0	1	2	5	81	95
Per cent affected with anthracnose	0	0	0.3	0.2	0.3	41	36

While observations were made each year as to the possibility of infection of seedlings from overwintered vines, no further definite efforts were made to determine this point until the fall of 1915, when a series of experiments was planned with this end in view. In these experiments the beans were planted in soil in ordinary greenhouse flats. Each flat was numbered, and the conditions involved in the case of each are given herewith. Seeds from healthy pods were used for planting each flat except flat 2, for which seeds spotted with anthracnose were used.

Flat	Description
1	Clean soil
2	Clean soil. Seeds spotted with anthracnose used in planting
3	Contaminated soil
4	Contaminated soil sifted to remove particles of bean tissue
5	Clean soil. Diseased vines and pods from crop of 1915 obtained from disease garden, broken up and mixed with soil in flat
6	Clean soil. Diseased vines and pods from crop of 1915 obtained from disease garden, placed on surface of soil and removed as seedlings were appearing
7	Clean soil. Diseased vines and pods from crop of 1915 obtained from disease garden, placed on surface of soil and left there
8	Clean soil. Diseased vines and pods from crop of 1915 kept in seed house, placed on surface of soil and left there
9	Clean soil. Diseased pods from crop of 1916 kept in seed house, placed on surface of soil and left there

The clean soil (uncontaminated by *C. lindemuthianum*) used was taken from a field where no beans had ever been grown so far as was known, but it was not sterilized except in the last experiment, when it was thought to be contaminated with spores. The flats, unless new, were sterilized with steam. The contaminated soil came from places in the disease garden where bean plants badly affected with anthracnose had grown in 1915. Only the surface soil to a depth of from two to four inches was used. In the first experiment both sifted and unsifted soil was used, but thereafter all contaminated soil was passed through a rather fine sieve in order to remove particles of bean-plant tissue as far as possible.

The healthy seed used was selected from clean pods, and in all but two experiments (II and III) it was immersed for three minutes in a 1–1000 mercuric chloride solution and afterward washed in tap water. This was done to destroy any spores of *C. lindemuthianum* that might possibly be present. The affected seed was disinfected in the same way as was the healthy seed. Seed so badly diseased that it could not germinate was not used. The affected seed used in each experiment was of the same variety as the healthy seed used. Davis White Wax was used in all experiments except Experiment IV, in which Refugee Wax was used. The old vines and pods affected by anthracnose were of several varieties and no attempt was made to keep them separate. Most of them were well covered with anthracnose lesions. Care was taken to remove all seeds, even shriveled ones, from the pods. The vines and the pods left in the garden over winter were much overrun with numerous saprophytic fungi and were somewhat decomposed by spring. Those kept in the seed house were as intact as they were when placed there.

In setting up an experiment, for example flat 6, the soil was first placed in the flat, the seed was then disinfected and planted usually in four or five rows to a flat, the vines and pods were placed over the surface to a depth of about one and one-half or two inches, the flat was placed in the greenhouse, and tap water was sprinkled on it until the soil was saturated. Unused sphagnum was placed over the flats unmulched by pods or vines in the case of Experiments V and VI. Each flat was prepared separately and every precaution was taken to avoid contaminating one with another.

The temperatures at which the flats were kept in the different experiments varied considerably, but when possible they were kept between 65° and 70° F. This was done in order to favor the fungus and permit infection to take place, and for this reason also the flats were watered more profusely than is best for good germination of bean seed. As a result the germination was very poor in some instances.

Records giving the extent of affection are not included here as they are all very much alike. On nearly every plant the stem was affected with one or more lesions, and often these occurred only at the surface of the ground. A few plants had lesions on the leaves. Lesions on cotyledons coming from the spotted seed used in flat 2 were not considered. The records were usually taken in about three weeks or less after the experiment was set up, in order to get them before secondary infection occurred. There is no doubt that secondary infection did occasionally occur.

These experiments, the results of which are shown in table 6, cannot be regarded as ideal. The results obtained in some cases — for example, from flat 1 in Experiment II — may seem to cast doubt on their reliability. But when the entire series is considered, there can be no doubt that the results show the possibility that old bean pods and vines serve as a source

TABLE 6. RESULTS OF A SERIES OF EXPERIMENTS TO DETERMINE WHETHER CONTAMINATED SOIL OR ANTHRACNOSE-AFFECTED VINES AND PODS OF VARIOUS AGES CAN SERVE AS A SOURCE OF INOCULUM TO BEAN SEEDLINGS

Experiment, and date when set	Flat	Number of plants appearing	Plants affected	
			Number	Per cent
I Nov. 24, 1915	1	12	0	0
	2	47	12	26
	3	24	0	0
	4	35	16	46
	5	24	19	79
	6	17	15	88
	7	14	14	100
II Dec. 29, 1915	1	44	11	25
	2	26	16	62
	4	45	0	0
	5	47	31	66
	6	24	21	88
	7	48	28	58
III April 28, 1916	1	35	0	0
	2	33	16	48
	4	41	0	0
	5	32	2	6
	6	25	5	20
	7	30	4	13
	8	18	15	83
IV June 7, 1916	1	38	0	0
	2	22	21	95
	4	37	0	0
	5	40	2	5
	6	39	1	3
	7	39	35	90
	8	38	34	89
V Nov. 10, 1916	1	34	0	0
	2	26	22	85
	8	31	31	100
	9	38	38	100
VI June 13, 1917	1	8	0	0
	2	17	9	53
	8	12	6	50
	9	72	34	47

of inoculum for the infection of bean seedlings. Apparently soil that is devoid of affected bean tissue cannot serve as a source of inoculum for more than two or three months, or is not an important factor in bringing about infection even though a few spores may live over winter in it.

In order to determine whether old affected vines and pods can serve as a source of inoculum under conditions prevailing out of doors at planting time in the spring, Davis White Wax beans were planted on June 10, 1916, in clean soil in the disease garden; that is to say, in soil in which beans had not been grown since 1911, at which time they were badly affected with anthracnose. Four rows, with forty hills to a row and five seeds to a hill, were planted in the following manner and with the following results (recorded June 26):

Row 1. Seed spotted with anthracnose planted. No mulch used. Only fifteen plants in six hills were alive, all of which showed affection with anthracnose.

Row 2. Clean seed planted, and hills mulched by placing over each a handful of old affected vines left outdoors in a pile over winter. One hundred and sixty-five plants appeared, every one affected on stem or leaves or both.

Row 3. Clean seed planted, and hills mulched by placing over each a handful of old affected vines kept dry in seed house. One hundred and seventy-one plants appeared, every one affected on stem or leaves or both.

Row 4. Clean seed planted. No mulch used. This row was about a rod north of the others. One hundred and sixty-nine plants appeared, on which no infection was observed at this time.

The weather from June 10 to June 15 was clear and warm most of the time and was favorable for seed germination. From the evening of June 15 to June 21 inclusive it was cool and rainy or cloudy, weather very favorable for infection to take place. From June 22 to June 26 inclusive it was clear and warm.

These results plainly indicate that under favorable conditions in the field, affected bean pods and vines from the crop of two preceding years can serve as a source of inoculum when they come into contact with seedlings of a susceptible variety of beans. Nevertheless it is probable that but little infection takes place from contaminated soil or from overwintered vines that are plowed under in preparing the land. In these experiments special pains were taken to place a considerable amount of badly affected bean tissue where it would be in continued contact with the seedlings. In ordinary practice this would occur only accidentally if at all. But when considerable wet weather follows planting, infection may be expected to occur from contact with old affected vines spread with manure on the surface of the soil after plowing, or from such affected vines as come to the surface after having been plowed under.

Muncie (1917:15–21) carried on tests to determine whether the bean-anthracnose and bean-blight organisms are able to winter over in old bean trash containing affected vines or as spores in the soil. He observed during the summer a small amount of anthracnose on the plants grown in pots where spores had overwintered and where old anthracnose-spotted vines of the second as well as the next preceding year had overwintered. A greater number of plants became affected with blight in these trials. From these data Muncie is convinced that the causal organism of both anthracnose and blight can live over winter in the soil in diseased bean trash and as spores or bacteria in the soil, and infect the crop of the following season. His field observations of preceding years doubtless strengthen this opinion.

Schaffnit (1920) obtained spores with unimpaired germination up to February 12, 1920, from pods of the harvest of the preceding year. Meyer (1910) finds the first appearance of the disease always on plants growing on damp ground directly where fresh stable manure has been applied but not where commercial fertilizers have been used. Pfeiffer (1910), however, believes the organism does not come from manure, as he has observed the disease commonly on land that never receives it. Muncie (1917:21) records inoculations made with an inoculum consisting of dung from a cow fed with bean straw infected by *C. lindemuthianum* and *Bacterium phaseoli*, but states that he was unable in this way to produce anthracnose and that blight appeared on only one plant. It is not unlikely that beans planted on heavily manured ground would produce so rank a growth that moisture conditions about them would be more favorable for infection than with plants not so vigorous. It is also possible for the pathogene to be introduced into a field with manure having infected bean straw mixed with it.

Further infection of the plant and spread of the disease through the field

The inoculation of the leaves, the petioles, the branches, and the inflorescence of the older plants occurs in the same manner as that of the seedlings. The plants, being larger, are more likely to be in contact with one another and thus make easier the transmission of the spores. If the vines have a tendency to hang low and be bushy, the air beneath them remains sufficiently humid for spore germination for a longer time after rains and dews than does the air outside. During rains the drops of water on the plant run together and move over the surface, in some places dripping from the leaf apices to other leaves and vines, in other places running down the stem or falling in large drops on the pods, where they spread out over the surface as they strike. When wind accompanies a storm, the wet leaves are blown against one another, against the leaves and stems of other plants, or to the ground. If a plant is affected with anthracnose, multitudinous spores from the various lesions become suspended in the rain water and are carried as described to the healthy parts of the plant. The pods become inoculated in this manner and in other ways.

When falling raindrops strike a spore-producing lesion or a film of water anywhere containing spores, drops of the contaminated water are spattered to the near-by stems and leaves. Faulwetter (1917), in his efforts to explain the rapid dissemination of *Bacterium malvacearum*, has made some interesting experiments to determine the distance to which water can be splashed by falling drops of various sizes. He finds that splashing occurs only when drops fall on a film of water, and that it is the water of the film that is splashed. A drop 0.1 cubic centimeter in volume falling 16 feet onto a film over a glass plate spattered water as far as 64 inches. A drop 0.02 cubic centimeter in volume falling 16 feet during a wind of 10 miles an hour "splashed water in abundance a distance of 8 feet......in moderate quantities as far as 12 feet...... and in slight amounts to 16 feet." He concludes that the possibilities of dissemination of bacteria in suspension in film water on cotton leaves " are considerable if one includes the distance bacteria may be carried from the original lesion, then splashed up again and carried farther, and so on, until a dilution too great for infection is obtained."

In addition to being splashed from contact with falling raindrops, contaminated drops of water may be carried bodily during a heavy wind to healthy parts of the plant, and plants blowing against one another will aid in disseminating the spores. Persons, as well as dogs, rabbits, pheasants, and other animals, passing through the field when the plants are wet, may serve to carry the spores from one plant to another. Insects, although minor agents, no doubt aid in inoculating healthy plants.

Gardner (1918:34) has shown conclusively that after a rain the spores of *Colletotrichum lagenarium* are present in the soil beneath affected plants, and that they may be disseminated by the spattering of such soil during a rainstorm. He has shown also that spores may be carried across a field in surface water during storms and washed or spattered onto healthy plants, infecting them and thus greatly enlarging the foci of infection.

No field observations of the spread of the bean-anthracnose disease were made during the three years when the writer's field work was done, since the disease appeared to only a slight extent during that time. What has been said regarding the spattering of contaminated water and soil in the dissemination of the two diseases mentioned would seem to be true in the case of *C. lindemuthianum.* Certainly the similarity of this organism and *C. lagenarium* in the matters of spore production, character of spores, and method of infection, would lead to the conclusion that the means of dissemination and inoculation shown in the case of the cucumber-anthracnose organism would exist also in the case of the bean-anthracnose fungus.

Infection of seeds

The appearance and extent of the canker on the seed, and its progress from pod to seed, are described on pages 107 and 111. As the pod matures, the seeds within prepare for a resting period. The cells, now packed with reserve food, become denser, drier, and more capable of resisting decay. The mycelium of the fungus within the cells of the seed also becomes less active, and possibly entirely dormant. It renews its growth, however, at conditions of temperature and humidity to which the embryo of the seed responds but slowly. There is probably very little activity of the fungus in seed stored in a dry place. Halsted (1892:285) and others report having found viable spores on and within spotted seed stored in such a place, and the writer has often so found them. The acervuli were black and dry, and it was not ascertained whether spores were being produced under such conditions or whether they were produced earlier when the seed was in a more moist condition. Just how long the fungus can live in old seeds has not been determined, although cultures were obtained from seeds two years old.

Infection studies

Susceptibility of plants of different ages to infection

But little evidence has been presented by investigators concerning the susceptibility of plants of different ages to infection. Frank (1883 a: 34) and others find that young and half-grown pods are more susceptible than older ones. Edgerton (1908:397) remarks that the young bean plant is not readily infected, but his later work (1910:41) indicates that he had but little trouble in securing infection on seedlings. Cook and Taubenhaus (1912: 49), noting a correlation in this first statement of Edgerton's with results which they had obtained on young fruits of apple and pear with several Gloeosporia attributed by them to the presence of an enzyme capable of forming a tannin-like body toxic to fungi, tested the oxidizing power of the enzyme present in the bean plant at various stages of its development and found that the oxidizing power is greatest in the young bean plant, is small during blossoming and the formation of young pods, is large again when the plants have large green pods, and decreases again when the pods are yellow. After making numerous inoculations on plants at all stages of growth, the writer is convinced that a young bean plant is more susceptible than an older one, and that young parts are more susceptible than older parts. An experiment was made in 1910 in which Refugee Wax beans were planted at intervals of seven and eight days throughout the season, beginning on June 27 and ending on August 22. In the evening of August 31, three days after the beans last planted appeared, all plantings were inoculated by spraying a suspension of spores over all parts of the plants. During the following day the plants were protected as far as possible from drying out by placing wooden boxes over the hills. When observed six days later, all the seedlings were badly infected and some were dying. Good infection was obtained on the younger leaves and petioles of beans planted one month earlier, but slight infection on the older leaves. Those planted two months earlier and having some mature pods were only slightly affected on leaves and vines, but good infection was obtained on young pods. The nearly mature pods showed no evidence of infection. The writer has always obtained good infection on pods of susceptible varieties when inoculated under favorable conditions for infection before they passed their brittle, or "snap," condition.

Susceptibility of different parts of the plant to infection

As mentioned in the preceding paragraph, Frank (1883 a) says that young bean pods are more subject to attack than are older pods, and this has been noted also by other early writers. Frank further found the pods to be more susceptible than the stems, the leaves, and the petioles, although inoculation on young bush beans resulted in the appearance of lesions on the hypocotyl, the epicotyl, and the veins of young leaves. He found the cotyledons especially susceptible. Scribner (1888) states that parts of the plant other than the pods and the seeds are rarely attacked. Edgerton (1910: 42) found that when bean seeds were inoculated with spores of the fig- and the cotton-anthracnose fungi, these organisms were able to attack and grow in the semi-living cotyledons but did not infect other parts of the plants. However, the cotyledons are to be regarded rather as culture media than as host tissue. Lauritzen (1919: 33) says the secondary leaves of the bean are more pubescent and seem to be affected more easily at the limiting humidities. This seems to be due to favorable humidity rather than to susceptibility. Others, in writing on the subject, have usually described the disease as occurring on the leaves, the stems, the pods, the seeds, and the cotyledons. The writer has found all young parts of the plant, including the roots, subject to attack, but as they become older they grow more resistant. The tissues of the seedling and of the young pod appear to be the most susceptible. The epicotyl of young bean plants of varieties showing marked resistance to the disease has often become affected when the plant was inoculated, although the other parts showed no lesions. In such cases, resistance may be due to the inability of the infection tube to penetrate the cuticle.

Susceptibility of different varieties of Phaseolus vulgaris L. to infection

In a number of the treatises on bean anthracnose, certain varieties are mentioned as being more susceptible to the disease than other varieties, but in most cases no inoculation experiments have been conducted to confirm these statements. Frank (1883 b: 511) says that Lindemuth observed the disease on the red-mottled Zucker-Stangenbohnen while other varieties near by were not affected, but a few years later he found many varieties susceptible. Trelease (1885), in speaking of the "peculiar susceptibility of the white-podded bean," says that "it is not improbably connected with the delicacy of its tissues, which selection has produced." Scribner (1888) states that wax, or butter, beans — those having yellow pods — are the kind most subject to attack, and later (1889) finds that no variety is exempt, not even those with green pods. McCarthy (1892: 14) says that wax varieties are especially susceptible. Harvey (1894) finds the disease worse on white-podded bush and pole beans. Cobb (1894:379) says that all varieties of *Phaseolus vulgaris* are attacked, though the tenderness of the wax (or butter) beans makes them more susceptible. Halsted (1898:312), after growing six varieties of beans, finds Green Flageolet the most susceptible to anthracnose and blight. Whetzel (1906) states that probably all the "rust-proof" varieties placed on the market will spot under conditions most favorable to the fungus. Tracy (1907), in his notes on varieties, records considerable variation in their susceptibility to anthracnose. Jarvis (1908:162) finds that, while wax-podded varieties are more susceptible, some desirable green-podded beans also are affected. Edgerton (1910:49) finds a number of wax varieties with which he has been working to be very susceptible. He finds Valentine somewhat resistant, and Nox All highly resistant on leaves and stems; and he speaks of Hodson Wax as having a reputation among growers for resistance, although he finds it somewhat susceptible. He thinks that observations of the amount of anthracnose on different varieties growing side by side is of little value in determining their resistance, and believes that the only way to test their resistance in a satisfactory manner is to inoculate each variety with the spores of the fungus. Ferraris (1913) lists a few very susceptible and a few resistant Italian varieties.

The writer (Barrus, 1911) has inoculated a large number of varieties of *Phaseolus vulgaris*. At the time when that work was done, no variety tried proved entirely immune to all cultures. However, it was discovered that there are at least two forms or strains of the organism physiologically different from each other although morphologically and culturally alike. Certain varieties of beans resistant to one of the strains were susceptible to the other, and those resistant to the latter strain were often susceptible to the former. There were other varieties that were susceptible to all strains, but none were found that were resistant to all. Edgerton and Moreland (1916) inoculated twelve varieties of *P. vulgaris* with cultures of the pathogene from eleven sources and obtained somewhat similar results. The snap varieties generally were susceptible to all cultures, while the Scarlet Runner and a variety which these authors call " Large White Kidney " were resistant to all. Three distinct types of cultures were found among those tried: one able to infect all varieties except the two mentioned; a second that affected the Boston Pea and snap beans but was not able to affect the Red Kidney to any extent; and a third that brought severe infection to the Red Kidney, moderate infection to the snap beans, but only slight infection to the Boston Pea. Fischer (1919:254) records the observations of several German writers concerning the comparative resistance of the different varieties grown in Germany. Some are inclined to think that the wax varieties are more susceptible, others that green snap beans are. Several have observed that pole varieties are more resistant than bush beans, but Fischer says the greater freedom of the former from anthracnose is due to their position on poles, where the conditions are not so favorable for infection as with vines near the ground. He is inclined to believe that the contradictory observations show the existence of different strains of the organism in the various localities. These observations, and those made by him on twenty varieties planted so as to become infected from spotted seed planted among them,lead him to believe that there is no variety in Germany resistant to anthracnose at all times and under all conditions. The most resistant variety of all appears to be Ideal Wachs-Buschbohne. Schaffnit (1920) observed the relative degree of infection on forty-five varieties of beans during 1914 and 1915 at six different localities in Germany, and lists a number of German varieties under each of the following headings: least attacked, moderately attacked, badly attacked. In general he found that varieties descended from the stem "Flageolet" are the most affected, while those descended from Hinrichs Riesen are the least affected; also, that pole beans show much greater resistance than do bush beans. This suggested to him the possibility of crossing pole and bush beans in order to obtain a resistant bush variety. This work he had not completed at the time when he prepared his paper.

The writer (Barrus, 1918) reports the results of a large number of inoculations made on many varieties of *P. vulgaris* and of other beans, with cultures from fifteen different sources. These for the most part fell into two physiological forms or strains, which are designated, respectively, as *alpha* and *beta*. The varieties of beans, from their behavior when inoculated with these strains, showed a classification into four groups, as follows: ab, those susceptible to either strain of the pathogene; Ab, those resistant to *alpha* but susceptible to *beta*; aB, those susceptible to *alpha* but resistant to *beta*; and AB, those showing resistance to both *alpha* and *beta*. In the first-named group, ab, belong 80 per cent of the wax bush varieties inoculated, 50 per cent of the green-pod bush, 40 per cent of the wax pole beans, and 30 per cent of the green-pod pole beans. It appears from this, then, that the wax bush beans are, generally speaking, the most susceptible to anthracnose. Five varieties are placed in the resistant group AB, but of these only one, Wells' Red Kidney, has shown a satisfactory resistance under all conditions. The writer (Barrus, 1915) described this strain of Red Kidney as having been selected by a farmer as a single healthy individual from a field where all other Red Kidneys were badly affected. This strain has continued to show the same resistance in the many inoculations made by the writer and by other investigators. It is not a pure type, however, the seed varying somewhat in size, shape, and color, and individuals appear from time to time which show some susceptibility to anthracnose, varying from slight to considerable. In a later paper (1918:602) the writer records the resistance of a field bean called White Imperial to several inoculations made with both strains of the pathogene. Recently, however (in 1921), a field of beans said to be White Imperial, the pods of which were severely affected with anthracnose, was observed. It seems not unlikely that strains of the pathogene exist which are capable of infecting even these varieties.

Burkholder (1918:353) has been able to produce an anthracnose-resistant White Marrow by crossing the ordinary susceptible White Marrow with the resistant Wells' Red Kidney and by subsequent selection of the resistant types. In the same manner, McRostie (1919:141) has also reported crossing the Wells' Red Kidney with the Michigan Robust, a white pea bean, but progress had not been made beyond the F_2 generation at the time of this report. In both of these crosses the character of resistance proved to be dominant, and inoculation of the F_3 generation is necessary in order to determine which individuals are homozygous and which are heterozygous to resistance.

No continued efforts have been made, so far as the writer is aware, to render susceptible bean plants immune to anthracnose by the absorption of toxic substances, as has been attempted with some other plants. The writer has endeavored to obtain a greater degree of susceptibility in bean plants showing considerable resistance to one of the strains of *Colletotrichum lindemuthianum.* Such plants, while yet seedlings, were, just previous to inoculation, (a) kept growing in very dry soil for two weeks, (b) kept in very wet soil for two weeks, (c) injured in various ways. In no case was a greater degree of infection obtained than on plants not so treated. Schaffnit (1920) remarks that a weak condition of plants such as comes from a sudden check in growth, as during a sudden fall in temperature, makes them especially susceptible.

Susceptibility of other species of the genus Phaseolus to infection

The statement has sometimes been made that varieties of *Phaseolus vulgaris* only are susceptible to attack by *Colletotrichum lindemuthianum.* Massee (1898), however, reports the disease as most common on French beans (*Phaseolus vulgaris*) and on Scarlet Runner (*P. multiflorus*), and a few other writers on the subject have recorded it as being severe or as occurring on Scarlet Runner (*P. multiflorus*), possibly quoting from Massee (Saccardo 1898, Collinge 1911, Lind 1913). Allescher and Schnabl (1893) distributed specimens of *Gloeosporium lindemuthianum* on leaves of *Phaseolus multiflorus.* Lakon (1916) says that *P. multiflorus* is practically resistant to rust and above all to anthracnose. Schenk (1917) also says that, although the disease is reported as occurring on Feuerbohnen (*P. multiflorus*) in Holland, he has observed this species to be very resistant when other varieties were badly affected. Fischer (1919:258) saw nothing of the disease worth mentioning on three varieties of *P. multiflorus* — *albiflorus* Lam., *coccineus* Lam., and *bicolor* Arrabida—while pole beans of *P. vulgaris* growing next to them were badly affected. Edgerton (1910:41) reports a slight infection of lima beans (*Phaseolus lunatus* var. Small White Pole), and later (with Moreland, 1916:8) good infection on Fordhook's Bush Lima, very slight infection on Large White Kidney, and no infection on Scarlet Runner when inoculated with spores of *Colletotrichum lindemuthianum.* Lind (1913) records the disease on pods, stems, and leaves of *Phaseolus vulgaris*, *P. compressus*, *P. nanus*, and *P. multiflorus.* The specific name *nanus*, however, refers to the bush forms of *P. vulgaris*, and the name *compressus* to another classification of varieties now included in *P. vulgaris*.

The writer has reported (Barrus, 1918:610) some good infections from inoculations made at various times during several years on the leaves of a number of varieties, both pole and dwarf, of *Phaseolus lunatus* L., both the small, or sieva, lima and the large lima. Fair infections were obtained on the stems, and also, from field inoculations, fair to good infections on the pods (fig. 15). From inoculations made at various times, the varieties Scarlet Runner and White Dutch Runner, of *P. multiflorus* Willd., have shown slight to fair infection on leaves and pods; the latter variety especially showed fair infections on pods (fig. 16) and seeds. The tepary bean, *P. acutifolius* var. *latifolius* G. F. Freeman, has always become badly affected when inoculated in the seedling stage.

The article just cited reports also the results of inoculations made on from one to several varieties of a number of species of Phaseolus supplied by E. V. Piper, of the United States Department of Agriculture. Slight infection was obtained on *Phaseolus aureus* Roxb., but on *P. aconitifolius* Jacq., *P. angularis* (Willd.) W. F. Wight, *P. calcaratus* Roxb., *P. mungo* L., *P. semierectus* L., and *P. sublobatus*, no definite infection resulted.

Susceptibility of plants of other genera and families to infection

Frank (1883 b:518) obtained no infection on cucumbers which he inoculated with anthracnose. Scribner (1888:362) states that the fungus attacks watermelon rinds as well as beans. Farlow and Seymour (1888) and Saccardo (1898:316) record it as occurring on *Citrullus vulgaris* Schrad. Galloway (1889) ascribes the disease "melon rust" to *Gloeosporium lindemuthianum* Sacc. & Magn. Halsted (1893 b:327, 329) obtained infection, from beans, on detached fruits of eggplant, pear, citron, pepper, and persimmon, and he believes (page 333 of reference cited) that the three solanaceous plants, tomato, eggplant, and pepper, are preyed upon by the same *Colletotrichum lindemuthianum* which causes a pod spot of the bean. Stoneman (1898:93) points out that inoculations made on detached fruits placed under a bell jar cannot be depended on, as such fruits are much less resistant to attack than are fruits under normal conditions, and are more in the nature of culture media. Smith (1904:28) obtained good infection from inoculations made on bean, but none on cucumber,

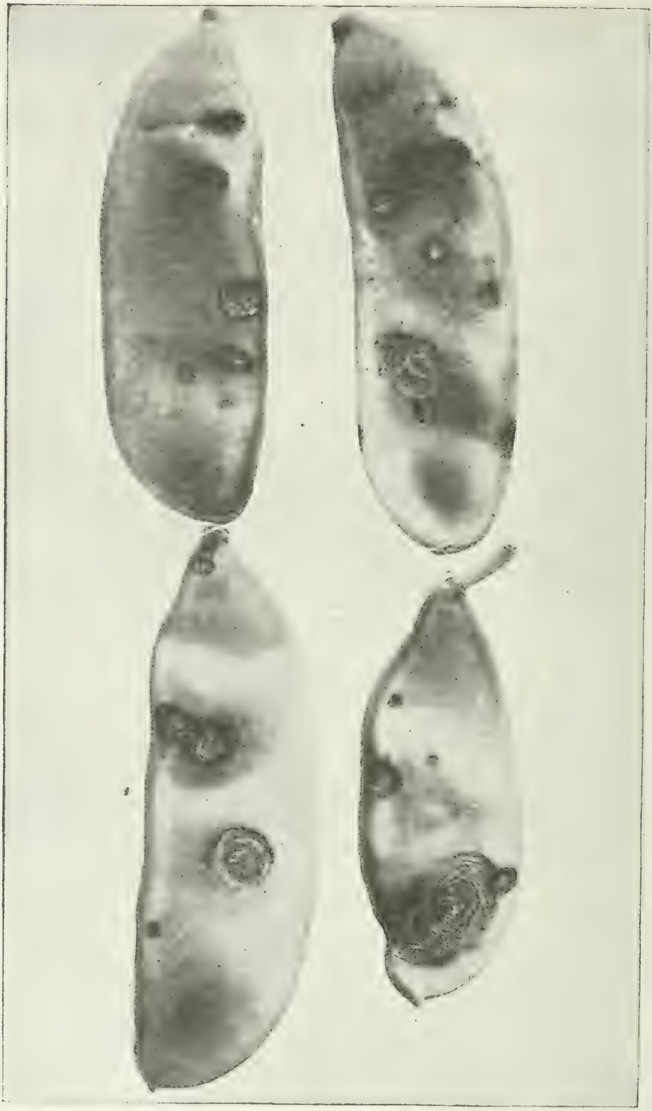

FIG. 15. ANTHRACNOSE ON PODS OF PHASEOLUS LUNATUS, LIMA BEAN

pumpkin, squash, watermelon, and muskmelon. Cook and Taubenhaus (1912:22, 27) obtained only negative results from inoculations of young and ripe apples and pears with *C. lindemuthianum.* Edgerton (1911:8) also was unable to infect apples with this organism from inoculations

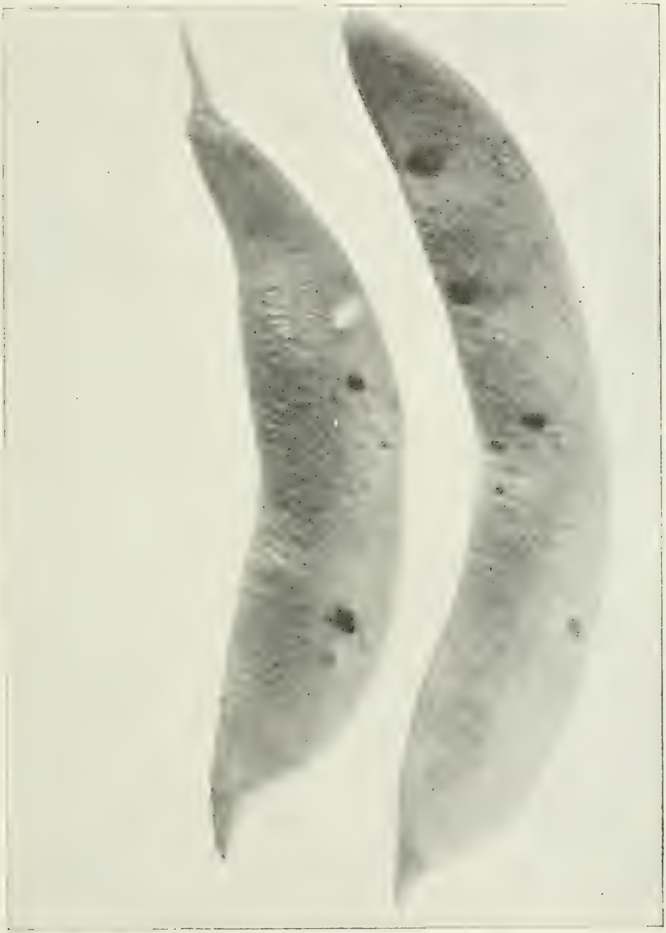

FIG. 16. ANTHRACNOSE ON PODS OF PHASEOLUS MULTIFLORUS VAR. WHITE DUTCH RUNNER, PRODUCED BY ARTIFICIAL INOCULATION MADE ONE MONTH BEFORE PHOTOGRAPH WAS TAKEN

which he made on picked fruit kept in a moist chamber. He was unsuccessful also in his attempts to infect the leaves, stems, and fruit of cucumbers with this fungus (Edgerton, 1910: 41). Shear and Wood (1913:78) inoculated fruit of apple, young wax bean pods, young cotton bolls, a mature pumpkin, a mature squash, green tomato fruits, and a nearly mature watermelon, with the conidia of the bean-anthracnose fungus, punctures being made in all except the bean pod, in which case the spores were applied to the surface; but in no case, not even with the beans, was infection obtained. It is possible that the beans inoculated were resistant to the strain of the pathogene employed. Krüger (1913:294) inoculated beans and cucumber fruits kept under the same bell jar, getting good infection on the beans but none on the cucumber even when it was injured, and he concludes from this that the forms occurring on the two plants are different. Inoculations which he made on apples, bananas, and tomatoes were unsuccessful on the first two, although they resulted in a whitened, sunken, spore-producing area about the punctures.

Chester (1894) reports that many seedlings of cowpea (*Vigna sinensis* Endl.) of the Conch variety died as a result of an attack of anthracnose caused by *Colletotrichum lindemuthianum*, and an examination of the seed showed that 9.5 per cent were diseased. McCarthy (1894:151) reports that pods of cowpeas nearly full-grown were occasionally attacked by a species of Gloeosporium. Butler (1918:262) reports an anthracnose on cowpea in India, saying that in general its characters agree with that of the bean but that experimental work is necessary before the two diseases can be pronounced identical. He records also (page 267 of reference cited) an anthracnose on val (*Dolichos lablab* L.) and on kulthi (*D. biflorus* L.), and tentatively refers the causal pathogene to *Glomerella lindemuthianum* (Sacc. & Magn.) Shear.

Sydow (1886) distributed what appear to be diseased pea pods (labeled *Vicia sativa*) under the name *Gloeosporium lindemuthianum*. Viala and Pacottet (1905) refer to *Colletotrichum lindemuthianum* as the fungus causing anthracnose of peas and beans, and Cooke (1906) reports it as being the cause of a disease of peas as well as of French beans. Other writers have not reported it on peas, and Edgerton (1910:41) could not secure infection from inoculations made on garden peas even after the wax was rubbed off the leaves. Fischer (1919:259) says that both Lemke and Feldt report having observed plants of *Vicia faba* severely attacked by this fungus, but his own observations do not confirm this and he thinks the disease observed was due to some other fungus.

Edgerton (1910:40) also sprayed spores of *C. lindemuthianum* on young bush beans, alfalfa, and cotton plants protected by bell jars, but obtained infection on the beans alone. Bäumler (1888) reports *Gloeosporium lindemuthianum* as occurring on stems and leaves of *Orobus vernus.* It seems that in this case the species of the pathogene was incorrectly determined.

The writer, in the course of his infection studies with beans, has inoculated seedlings of sweet pea (*Lathyrus odoratus* L.) and garden pea (*Pisum sativum* L.), vines and young pods of cowpea (*Vigna sinensis* [L.] Endl.), and young plants of mandrake (*Podophyllum peltatum* L.), with spores of *Colletotrichum lindemuthianum.* Excellent infection was obtained on beans used as checks, but the other plants remained free from disease. In a series of other inoculation experiments on seedling plants, fair to good infection resulted on the stems of certain individual black-eyed beans or cowpeas (*Vigna sinensis* [L.] Endl.), and slight to fair infection on the epicotyl of the kulthi bean (*Dolichos biflorus* L.); but no infection resulted on any part of the bonavist bean (*Dolichos lablab* L.), the guar bean (*Cyamopsis tetragonoloba* Taub.), the asparagus bean (*Dolichos sesquipedalis* L.), the jack bean (*Canavalia ensiformis* [D.] DC.), four varieties of horse bean (*Vicia faba* L.), and the garbanzo bean, or chickpea (*Cicer arietinum* L.). There are evidently but few species outside the genus Phaseolus which are susceptible in any degree to anthracnose, and no plants except varieties of *Phaseolus vulgaris* are susceptible to such an extent that the disease becomes epiphytotic in regions where such plants are extensively grown. It is not unlikely that several strains of the pathogene exist, one capable of infecting *Phaseolus multiflorus* or some of its varieties in a severe manner, another able to attack *Vigna sinensis*, and others attacking plants related to the bean, each being confined rather closely to the species, or even to certain varieties within the species, to which they have adapted themselves.

Susceptibility of Phaseolus vulgaris to infection from other anthracnose fungi

Frank (1883 b:518) was the first to inoculate the bean with spores of another anthracnose fungus in order to determine whether the forms occurring on different hosts were really the same species. He sowed on green bean pods the spores of *Gloeosporium castagna* Mont., which had proved to be very virulent on the leaves of the silver poplar, but obtained no infection. He therefore concluded that gloeosporial forms are independent species and have their own host plants. Southworth (1890:48) attempted unsuccessfully to infect bean pods with the hollyhock-anthracnose fungus, *Colletotrichum althaeae* Southw. (*C. malvarum* [A. Br. & Casp.] Southw.), but was successful with *C. lindemuthianum*. Halsted (1893 b:327, 329), in connection with his cross-inoculation work, obtained good infection on detached bean pods with *Colletotrichum* sp. from the eggplant, and with Gloeösporia from pepper, apple, and tomato; also (1893 d:248) with *Colletotrichum lagernarium* (Pass.) E. & Hals. from the watermelon. Sheldon (1904:132-135) inoculated wax-bean seedlings, bean plants in blossom, and bean pods on the plants, and also six different cucurbits, with spores of *C. lagernarium* from pure cultures obtained from a watermelon fruit. Infection was obtained on the cucurbits in most cases, but the beans for the most part were free from evidence of infection. Sheldon did find, however, spots bearing spores both on the beans inoculated by him and on those used as checks. He decides that it is not safe to conclude from his results that the anthracnose fungus on the beans is the same as that on the melons and the gourds. Edgerton (1910:41) obtained no infection on young bush beans sprayed with spores of the fig anthracnose (*Glomerella fructigena* [Clinton] Sacc.) and of the pepper anthracnose (*G. piperata* [E. & E.] S. & S.), and no infection on freshly picked bean pods placed in a moist chamber and inoculated with a culture of the rose-anthracnose fungus (*Gloeosporium rosae* Hals.); although in every case good infection was obtained with the bean-anthracnose fungus on the beans used as a control. Bean seeds wet with a suspension of spores of the fig- and the cotton-anthracnose fungus were somewhat infected. Spores of the fig-anthracnose fungus were being produced in abundance on seed that had rotted in the ground, and the semi-living cotyledons of the germinating beans were attacked by both fungi. The spots did not develop further, however, while in the case of seeds treated in the same way with spores of the bean-anthracnose fungus the cotyledons, and later the plants, became highly infected. Taubenhaus (1911:198) reports success in obtaining infection on pods of pole and bush lima beans in the field from puncture inoculation with spores of species of Gloeosporium from sweet pea, apple, and mandrake, or may-apple, and with

Gloeosporium officinale E. & E. from sassafras, *G. gallarum* Ch. Rich. from oak gall, and *G. psidii* Del. from guava. The disease produced, except in the last-named case, resembled sweet-pea anthracnose but did not resemble the bean disease. Shear and Wood (1913:77–89) inoculated detached bean pods with anthracnose conidia from avocado, cotton, guava, loquat, mandarin, orange, pitcairnia, and privet, but in the case of none of these did they obtain any infection. Krüger (1913) inoculated detached bean pods kept under bell jars with conidia of gloeosporial forms from apple, banana, and tomato, and, while good infection was obtained on checks consisting of detached fruits of the respective hosts, the result on bean pods was doubtful; no infection was obtained on the uninjured pods, although there was some mycelial growth in the tissue surrounding the injury made on other pods. Gardner (1918:23) inoculated five varieties of beans with *Colletotrichum lagenarium*, with negative results.

On inoculating bean seedlings of several varieties with spores of the anthracnose fungus from sweet pea and from mandrake, the writer did not obtain any infection. Fair infection was obtained on sweet-pea seedlings inoculated with spores of the form from sweet pea, and on the leaves and stems of young mandrake plants growing in the woods and of those transplanted to the greenhouse inoculated with the form from mandrake. From his own results and from those obtained by others, the writer is inclined to believe that, in general, each of the various anthracnose fungi is confined to its own host or to closely related plants.

ECOLOGIC ASPECTS

In the preceding discussion frequent reference has been made to weather conditions favorable to infection. There is no question that the weather is an important factor in the growth of this fungus, just as it is in the growth of higher plants. The fungus has its ups and downs according as the season is wet or dry. Temperature and humidity are the two factors of greatest importance, and of these the latter is usually the controlling one in the Northern States. This has been generally recognized by growers, and its significance in the development of the disease has been pointed out in some of the earlier accounts.

Edgerton (1910:28, and 1915:248) has shown that temperature is the controlling factor under conditions in Louisiana. The disease, while prevalent during the cooler part of the growing season, is absent from the fields there during the hot months of June, July, and August, even with ample rainfall and when diseased seed was used for planting. Furthermore, Edgerton experienced great difficulty in keeping cultures of the organism alive during the summer. The writer has never had any difficulty in obtaining infections from inoculations made during the summer, although for the success of the experiments he has made them at times when the conditions promised to be most favorable. Some of the inoculations were made in the greenhouse when the temperature during the following days ran high, and yet fair to good infection was obtained in several instances. Cultures of the fungus in the laboratory grow very slowly or not at all during the excessively warm weather of the summer, and in some cases they die when not transferred frequently. When placed in a cellar where the temperature remained at about 16° to 20° C., a good growth of mycelium and abundant production of spores resulted. During the summer season in the North, the periods of excessively high temperatures are short. The high temperature of the day is not sufficient to kill the fungus in the host, and growth and spore production may take place during the lower temperatures of the night. The effect of temperature on growth of the fungus in culture media has already been discussed (page 121). Lauritzen (1919:20), by employing inoculation chambers where temperature and humidity were controlled, determined the range of temperatures at which infection of bean seedlings takes place after inoculation with *Colletotrichum lindemuthianum*, when kept at a favorable constant degree of humidity, to be from 57° to 80° F. At the extremes the number of infections occurring are small, but they are more abundant at the more nearly optimum temperatures, as one would expect. Lauritzen says that the data point to a lower temperature limit for infection when forty-eight hours are used for the infection period than when twenty-four hours are used.

Moisture must be present or the humidity of the air high if the fungus is to gain entrance to the host. Lauritzen (1919:29) found that infection took place at humidities of 95.8 and above when the temperature was kept at from 65° to 68° F. It was not necessary that a film of moisture be kept on the leaf during the germination of the spore, for infection occurred when the plants were dried off after inoculation and before being placed in the inoculation chamber. Lauritzen did not determine how the spore gets sufficient water in such cases to permit germination, but he suggests that when the evaporation is not too great it may absorb water from the host plant by imbibition and later by osmosis.

If the soil is wet and cold, the bean seed germinates slowly, but the spores of the fungus when present on the seed under such conditions will germinate and penetrate the host before it appears above ground. If dry weather follows the appearance of the plants grown from spotted seed and infection has not yet taken place, the chances are good that the spotted cotyledons will drop off before any infection occurs. When this happens the plants are as free from infection as though they had come from healthy seed, but the affected cotyledons on the ground may serve as a source of inoculum for a time. Often after wet weather the juvenile leaves of the seedlings become very badly spotted. These in turn drop off, and if the plant has not been infected otherwise it may continue to develop entirely free of the disease (fig. 17). If, on the other hand, rainy weather should occur following the germination of spotted seed and before the cotyledons fall, some of the seedlings will surely become infected and the disease will be likely to spread to neighboring plants. In fact, an instance is known in which, of two fields planted from the same lot of seed but at different times, one field became badly diseased and the other remained healthy because rainy weather succeeded the planting of the one and dry weather the planting of the other.

If infection is to take place in plants inoculated with the spores of the fungus, a moist condition must prevail for at least eighteen consecutive hours at some time before the spores succumb to desiccation so that they may germinate and form appressoria. This statement is based on spore-germination tests recorded earlier (page 117). In the inoculation experiments conducted by the writer, the plants were usually kept in an inoculation chamber for about forty-eight hours, although successful results were obtained when they were left for but twenty-four hours. That the spores may endure a period of dryness before favorable conditions arrive was shown by an experiment in which seedlings growing outdoors were inoculated during the morning of a bright, hot day. The next morning, before the dew had evaporated, each plant was covered with a shaded lamp chimney plugged with cotton at the upper end. All these plants showed lesions after six days.

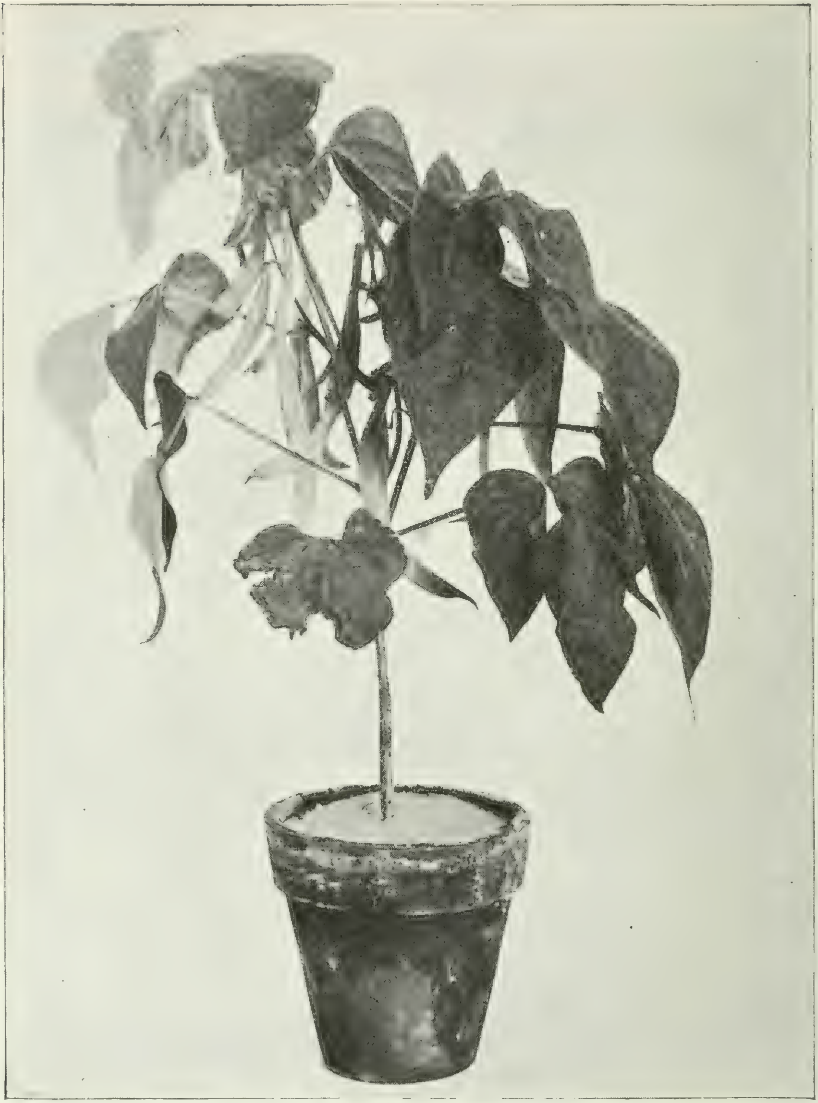

FIG. 17. BEAN PLANT FROM SPOTTED SEED, THE JUVENILE LEAVES AND STEM OF WHICH BECAME AFFECTED WITH ANTHRACNOSE BUT WHICH PRODUCED A CLEAN CROP OF PODS BECAUSE DRY WEATHER PREVAILED DURING THE SUMMER

(Transplanted to a pot for photographing)

A rainy period of several days accompanied by cool weather is the most favorable condition for infection and for the development of the fungus. Night dew or even humid air, however, is sufficient to allow infection to take place. Conditions permitting the plant to retain moisture beneath the vines are particularly favorable for the infection of the pods. Such conditions are afforded by poor soil drainage, or especially by poor air drainage such as may come about from a bushy growth of vines and from plants growing close together; and similar conditions may exist in a low area or pocket in the field.

Beans grown at the time of the year when the prevailing weather conditions are the least likely to be favorable for infection, will of course be the freest from anthracnose. Plants inoculated artificially during a rainy day will become badly diseased, while plants in neighboring hills in the same row will be but slightly affected and hills farther on not at all. This is because the weather has remained comparatively dry from the time of inoculation until the pods were maturing, and thus spore dissemination was prevented. The disease does not spread as readily through a field planted in check rows as through one planted in drills. Corbett (1907) says that growers of field beans find the disease worse on crops planted early. In New York State late-maturing beans are the most liable to damage from anthracnose, as weather conditions favorable for infection commonly occur during September. For this reason an early-maturing variety planted early would have a better chance of escaping infection, other things being equal, than a late-maturing one.

These facts explain why the disease materially decreased during the dry seasons of the four years from 1907 to 1910, and why it has been so severe during years of abundant rainfall. The seed produced in dry seasons is practically free from the disease, and when such seed is used the plants resulting therefrom are remarkably free from anthracnose, even though the season may be wet, because the source of infection is not present. After a season or so of rainy weather, however, the disease, at first inconspicuous, may become general throughout the field and a large amount of spotted seed may result. It has been claimed by seedsmen that in certain irrigated sections of the West where there is but little rainfall during the growing season, plants free from the disease can be grown from spotted seed; but it is questionable whether the moist conditions beneath the vines resulting from irrigation would not be sufficiently favorable for infection occasionally to take place, at least when spotted seed is planted. The disease is occasionally reported from such sections. Complete freedom from anthracnose may be expected in those regions where dry farming is practiced. Lauritzen (1919:32) suggests that the variation in the amount of moisture in the air in different regions may be an important factor in the distribution of diseases over the earth's surface.

PROPHYLACTIC ASPECTS

As early as 1893 a number of measures for the control of bean anthracnose had been recommended. Among these were such as treating seed with chemicals or hot water, selecting seed from healthy plants, spraying with bordeaux mixture and other substances, working among the plants only when they are dry, planting exclusively on ground that has not produced diseased plants the year before, and selecting a dry, airy situation with light soil. These recommendations, if carefully followed, will usually result in the production of plants free from anthracnose. Doubt has been cast, however, on the efficacy of certain of these recommendations, and it is worth while to consider the advantages and disadvantages of the various methods.

Seed disinfection

Seed treatment was first recommended by Halsted (1892:286), who at that time used ammoniacal copper carbonate. Later (Halsted and Kelsey, 1895:30) he tried full-, half-, and quarter-strength bordeaux mixture, and concluded that such treatment had but little effect in killing the mycelium within the seed. Beach (1892:320), after experimenting with hot water, bordeaux, ammoniacal copper carbonate, and potassium sulfide, found that, while hot-water treatment resulted in the lowest percentage of plants with anthracnose, the untreated seed gave a greater quantity of marketable beans because of the better stand, and that even with severe treatment, enough disease remained to injure the crop. Craig (1893), after soaking seed for varying lengths of time in solutions of different strengths of ammoniacal copper carbonate and copper sulfate, concluded that seed can be treated cheaply and advantageously with copper compounds, preferably ammoniacal copper carbonate. The best result he obtained, however, was but 79 per cent of healthy plants, and but 73 per cent of the treated seed germinated. Later (1898) he experimented with lysol, formalin, potassium sulfide, nitrate of soda, corrosive sublimate, and kainit. The lysol treatment resulted in 4 per cent of diseased pods, in comparison with 57 per cent in the check row. The value of other treatments are in the order named, corrosive sublimate giving 19 per cent of diseased pods and kainit 36 per cent. Bedford (1900) soaked bean seed for two hours in formalin solutions varying from one ounce in one gallon of water to one ounce in four gallons. The former dilution gave the better results, without the germination being affected appreciably. Sevey (1907) states that experiments at Cornell University, in which bean seed was soaked for forty-five minutes in formalin solution 1–200, gave profitable results. Kirk (1905) says that good results have been obtained by soaking seed for five minutes in water at a temperature of 140° F. (60° C.), or for fifteen minutes in water at 130° F. (54.4° C.).

Whetzel (1906) believes seed treatment to be of little practical value in controlling the disease, as such treatment reduces the stand and renders the seeds unfit for planting with a machine unless they are allowed to dry, in which case many of them slip their coats and thus become worthless. Fulton (1908:15) says that he has kept beans for forty-five minutes at 135° F. (57.2° C.) dry heat without appreciable effect on germination, while seeds placed in water during a like exposure are practically all killed. He thought that dry heat might be used as a seed treatment for control of bacterial blight, but experiments that he made to prove this gave negative results. Edgerton (1910:46–48), from experiments he has made, is inclined to believe that the disease may be materially reduced by soaking the seed for from ten to fifteen minutes in water at a temperature of 50° C. (122° F.), and that seeds thus treated will not slip their coats and their germinative ability is not impaired. Later, Edgerton and Moreland (1913) report that the treatment of bean seed with hot water 50° C. for eight minutes, with corrosive sublimate solution 1–1000 for twenty minutes, with benetol solution 1–50 for twenty minutes, or with corrosive sublimate 1–1000 in a 1–50 glycerin solution, did not materially reduce the percentage of germination below that of untreated seed, although formaldehyde solution 1–100 did reduce the germination in the field. However, none of the treatments except hot water had much merit as a control measure for anthracnose, although blight was materially reduced by the treatments with corrosive sublimate and benetol.

Hollman (1915:691) treated seed with 2.5- and 1.25-per-cent Chlorphenolquecksilber, with 1- and 2-per-cent sublimate, with 2.5- and 4-per-cent formalin, with a tar preparation called "Körnerschutz," with petroleum for thirty minutes, and with 2-per-cent copper sulfate, and also with 1-per-cent bordeaux sprayed on the seeds. None of these treatments were effective, the disease being serious in all plots. The Chlorphenolquecksilber seemed to have the effect of stimulating the plants to increased growth, so that they yielded more, in spite of the disease, than did plants in other plots.

Fischer (1919:249) discusses the results obtained by a number of investigators with seed treatment. He says that Appel used 1-per-cent sublimate; Remn and Vasters tried Chlorphenolquecksilber; Wahl tried a new and water-free Chlorphenolquecksilber called "Uspulun"; Weirup experimented with sublimate and later with Uspulun; Schander and Krause treated badly affected seed, which when untreated gave but 19.3 per cent germination, with 1-per-cent sublimate for fifteen minutes, resulting in 29.3 per cent germination; with 0.25-per-cent Chinosol for three hours, resulting in 32 per cent germination; and with 5-per-cent Uspulun for one and one-half hours, resulting in 46 per cent germination. In no case, however, was there a satisfactory control of the disease, which became general throughout all the plots. Even when an entire field at the experiment station was planted with treated seed — in which case infection could not have taken place from near-by plants from untreated seed — the disease appeared just as severely as in fields planted with untreated seed. Fischer concludes from these observations that seed treatment of beans to control anthracnose has no practical value.

Muncie (1917:26–37) treated seed for varying lengths of time with chemicals of varying strengths, and with both dry heat and hot water. The chemicals used were: mercuric chloride and sodium nitrate heated to 55° C. (131° F.) and not heated; mercuric chloride alone; mercuric chloride with oxalic acid; zinc chloride; formaldehyde solution as a dip and sprinkled on seed; formaldehyde gas; copper sulfate with the addition of sulfuric acid; and calcium hypochlorite. Both healthy seed, and seed affected with anthracnose and with blight, were treated, and the effect of the treatment on germination and on the pathogenes was noted. It is seen from the results given that in none of the treatments was either pathogene killed in all the seeds, except in those cases in which the treatment materially reduced the percentage of germination. Muncie states, in summing up the results of the treatments, that it seems possible to control the disease in a large degree in case of a light infection by employing seed treatments, and that the treatment most efficient is either a thirty-minutes immersion in a 35-per-cent solution of bleaching powder, or a sprinkling of the seed, as in the treatment of oat smut, with a solution of formaldehyde, using 1 pint of formaldehyde to 30 gallons of water. There is not sufficient evidence submitted in his data, however, to admit this possibility. His summary at the end of the bulletin states accurately the conclusions reached, namely, "Seed treatments with chemical solutions and wet and dry heat have failed to control these diseases." Rapp (1920) concludes from his numerous seed-treatment experiments with formaldehyde, mercuric chloride, sulfuric acid, dry heat, and hot water, to control bacterial blight, that no present method can be regarded as satisfactory, since in killing the blight pathogene the germinating power of the seed is either greatly weakened or totally destroyed.

Kidd and West (1918) soaked bean and other seeds for varying lengths of time (from six to seventy-two hours), and found that, while germination was more rapid from soaked than from unsoaked seeds, subsequent growth, particularly of the bean, was poorer — although with horse beans (*Vicia faba*) it was greatly improved. Bean seed was soaked in water at various temperatures, but always the resultant growth was poorer than when dry seed was used. The literature on the subject, however, reviewed by Kidd and West (1919), indicates a better growth and a higher total yield from soaking in a limited quantity of water.

Further experimentation with seed treatment should not be regarded as something bound to be resultless. No one has made a careful study of the permeability of the seed coat of the bean to various disinfecting agencies, nor of the action of these agencies on the fungus within the seed. Seed treatment, to be effective, must destroy the pathogene within the seed, notwithstanding the extent of its growth there, in order that the affected seed may not serve as a source of inoculum. At the same time, the treatment must not injure sound seed. Any treatment, to be of value, must achieve these two conditions, and the problem does not appear to be insurmountable. Nevertheless, healthy seed can be obtained in other ways. The selection of clean seed (page 168) certainly offers a greater promise of success.

Sorting out affected seed

The selection of clean seed has been recommended by many plant pathologists. Frank (1883b: 523), after having determined that infection of the seedling can take place from the fungus overwintered in affected seed, was the first person to recommend the use of clean seed by the sorting-out of spotted ones or by the securing of seed from non-infested fields. Beach (1892: 323–326) obtained good results by sorting out the affected seed before planting. Gain (1898: 200), after experimenting with Mont d'Or, a climbing wax-podded variety with dark brown seeds, states that he is able to discard practically every diseased seed by hand-sorting. He observes that diseased seeds are lighter than healthy ones, and advises growers to discard all light seed. In another article (1899: 389) he says that by carefully sorting out infected seed the anthracnose disease can be controlled. Whetzel (1908: 432), however, although he used the utmost care to remove every suspected bean, was unable to remove the diseased seed completely from white beans, a germination test of the seed thus sorted showing as many as 12 per cent of the remaining seed diseased. When an attempt is made to sort colored seed, an even larger percentage of diseased seed passes unobserved. Fischer (1919: 252) concludes from experiments conducted by Schander and Krause, and from other tests made at Bromberg in 1915 and 1916, that it is not possible to control the disease by this method since many affected seeds cannot be detected from their appearance.

It is, of course, impossible to sort out every affected seed even by the most careful examination. Such seed as are left in may produce affected plants if conditions following planting are favorable for the development of the fungus, and such affected plants may serve as a source of inoculum to other plants in the field. However, as every diseased seed discarded removes one source from the field, it would appear to be preferable to sort the seed carefully before planting rather than not to sort at all. A practical grower of a considerable acreage of beans in northern Vermont says that since he has made a practice of hand-sorting his seed he has had no anthracnose, but that before he did so the disease was harmful.

Selecting seed from clean pods

It has occurred to a number of experimenters that the surest way of obtaining clean seed is to select it from healthy plants or at least from unspotted pods. Harvey (1894) advises this method, and emphasizes the idea that pods showing any evidence of disease, such as pits, discolored patches, and wrinkled or blistered places, should be rejected. Cobb (1894: 383) recommends hand-picking the seed in the field, selecting only the healthiest-looking pods. Several others since then have recommended the practice. For several years Whetzel (1908:441) grew clean Black Wax beans that were selected from clean pods, and at a time when anthracnose was very prevalent throughout the State. He recommends selecting seed from clean pods, hand-picking them in the field, and resorting them later. He suggests also that seed from the irrigated regions of the West may possibly be free from the disease. A seedsman in an irrigated section of Colorado declares that he has been able to grow clean plants from diseased seed and could continue the practice without danger of the disease appearing in the field.

The writer selected seed from clean pods during four consecutive seasons (1908 to 1911), the purpose being to determine whether such seed would produce anthracnose-free plants with ordinary culture, particularly during a wet season, and whether such a method could be recommended as a general farm practice, Since 1911 he has been unable to carry out these trials further, except in a most limited way.

The seed for these experiments was obtained from pods picked in the field, or in a few cases from the mow. Any seeds showing evidence of anthracnose or blight were rejected. Later the pods were resorted, and, after shelling, the beans also were sorted to remove broken, undersized, or abnormal beans. The seed was then distributed, in lots of about a quart of each variety, to growers and experimenters in this and other States, in order to obtain, in some place at least, good conditions for a test. Those who received the seed were urged to plant it at a distance from other bean patches and to observe the precautions necessary to avoid communication of the disease to this seed patch.

In these tests extending over a period of four years, twenty-four different plots of beans, containing from one to eight varieties, were grown in various parts of New York State and in a few places outside the State. In five of these plots, there was some anthracnose on a few of the varieties. In all but one of these five cases the infection was slight, and in all cases the inoculum came from a source other than the seed.

The chief drawback to the success of the trials was the dry weather which prevailed during the growing season in these years. Beans grown from seed that had not been selected produced anthracnose-free plants in many cases. Plants grown by Dr. Edgerton in Louisiana from healthy seed sent to him were free from anthracnose and with few exceptions free from blight, while other beans grown in the trucking section were badly affected with both anthracnose and blight.

Another factor not given consideration at that time was the resistance of many of these varieties to at least one strain of the pathogene. It was learned later that this is true of the varieties Navy Pea, White Kidney, Red Kidney, Red Marrow, and Blue Pod Medium, and also of Turtle Soup to some extent. In most instances, however, anthracnose had in the past appeared in a destructive manner on the varieties sent for testing, in the localities under consideration.

The introduction of healthy seed of a certain variety into a locality in which the strain of the fungus to which it is susceptible is absent, would of necessity result in an anthracnose-free crop. This may be the reason why clean seed from other sections of the country often produces better yields than does home-grown seed. However, if affected seed of such a variety is introduced into a certain locality, the strain of the pathogene present on the seed, being capable of producing the disease in that variety, will multiply under conditions favorable for it and the entire crop may become diseased. Healthy seed is therefore always safer to use as long as the particular variety is susceptible to any strain of the organism.

In 1914 Professor F. C. Stewart, of Geneva, New York, planted seed that he himself had selected from clean pods. The plants remained healthy all summer and the pods were free from anthracnose when picked. A patch of Red Kidney and Turtle Soup beans selected from seed from clean pods in 1912 was planted at Ithaca at a distance of ten rods from other beans artificially inoculated with the two strains of *Colletotrichum lindemuthianum* during the summer. The plants from clean seed remained free from anthracnose.

In 1915 Bert Smalley, of Interlaken, New York, grew one row, fifteen rods long, of Red Marrow beans from seed selected from clean pods by Professor Stewart and afterward resorted by him in order to be sure there was no anthracnose present. The field in which this row of Red Marrows was grown was not cultivated at all during the season because of wet weather, and so weeds of various kinds grew up among the bean plants. On October 4 the plants were found to be generally affected with anthracnose. From the row 75 plants were selected, one plant at every pace. On these plants there were 209 pods, of which 137 were affected with anthracnose, 67 were not spotted, and 5 were doubtful. About 40 rods southwest of the end of this row was a large field of Red Kidney beans which were badly affected with anthracnose. It was concluded that the first infection of plants in the row planted with clean seed came from spores carried from the affected Red Kidneys by some animal, and, since the tall weeds about the beans kept them moist most of the time during the rainy season, and since they were planted in drills so that the plants were in contact with one another, the disease readily spread along the entire row.

These trials seem to indicate that the use of clean seed is not altogether successful in producing a crop of beans free from anthracnose unless the season is dry, under which condition there is no loss from the disease in any field. The experiments are reported because the results are such as may be expected in such cases. So far as observations were made in these and in other experiments, clean seed always produced anthracnose-free seedlings in the field, and this is all that can be expected of clean seed. Infection, when present, must have taken place from an inoculum of a source other than the seed. In some cases this came early from old affected vines or from near-by affected plants. Infection will always be likely to occur from such sources under favorable weather conditions unless precautions are taken to prevent it. Since clean seed can be depended upon to produce seedlings free from anthracnose, the question becomes one of deciding whether or not the presence of affected seedlings in the field is a sufficiently great menace to warrant the use of clean seed. The writer, from observations of his own experimental plots and of many fields in which clean seed as compared with affected seed has been used, is convinced that the presence of such affected seedlings is a menace that warrants the grower's going to considerable pains and expense in order to obtain healthy seed. Growers generally will probably prefer to buy clean seed if they can get it, rather than to take the trouble to produce it. But some one must produce it if it is to be had, and the question of production is the one concerned here. It is true that following dry seasons there will be but little difficulty in obtaining bean seed almost or quite free from anthracnose. But it must be possible to obtain such seed every year if the disease is to be avoided in years favorable for its appearance. The practice of selecting from clean pods is the only reliable way to secure seed free from anthracnose (figs. 18 and 19). The method in its practical application involves the use of a seed plot, on which the selected clean seed is planted. This plot should produce the necessary seed for planting the main field the following year. The steps in carrying out the measure are as follows:

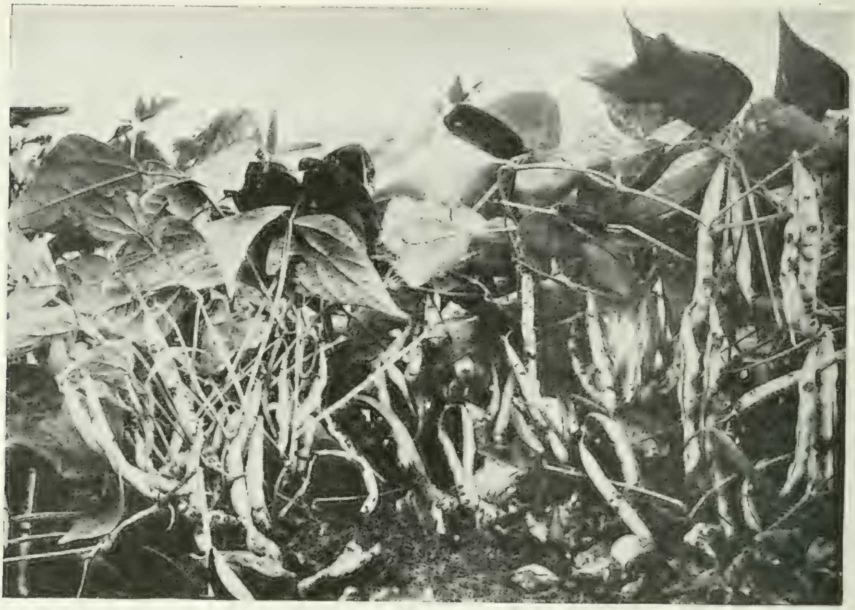

FIG. 18. A HILL OF WAX BEANS FROM SPOTTED SEED

The pods are so badly affected with anthracnose that they are worthless

1. Selection, in the fall, of pods having no anthracnose or blight spots. These should be shelled by hand or with a flail, and the seed afterward sorted over to remove all discolored, impure, undersized, shriveled, or broken beans. The amount of seed to be selected in this way will depend on the amount needed for planting the main field. If 40 bushels of seed are required for planting the main field and 20 bushels an acre can be

expected from the seed plot, then enough seed must be selected from clean pods to plant two acres.

When the main field contains 200 acres or more, the size of a plot necessary to produce sufficient seed for the main field will be so large that it will be difficult to obtain by pod selection enough seed to plant it. In such a case it is desirable to maintain an increase plot in addition to a small seed plot. The seed plot would be used to provide clean seed for the increase plot, which in turn would produce sufficient seed for the

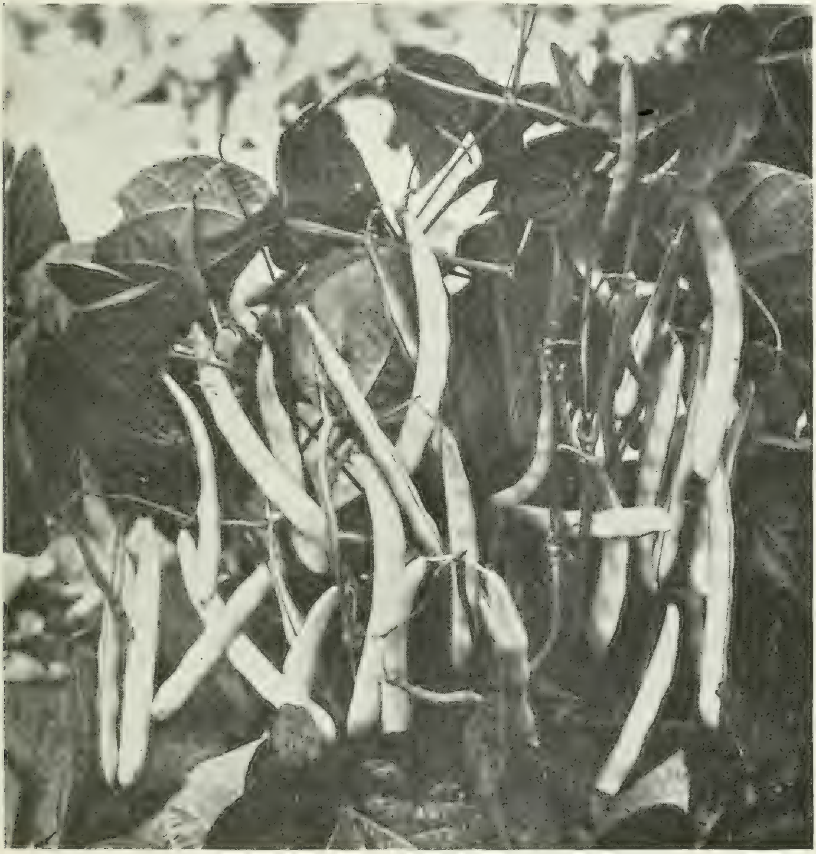

FIG. 19. A HILL OF WAX BEANS FROM HEALTHY SEED

The pods are entirely free from anthracnose main field. This plan would require two years to get seed for the main field, but would afterward permit an economy of time and effort.

2. Location of the seed plot at a considerable distance, preferably 40 rods or more, from other bean fields or garden patches containing beans. If an increase plot is used, it should be located at some distance from the seed plot and also at some distance from the main field. This is to prevent, as far as possible, the transfer of spores of the causal fungus from infected plants to plants in the seed plot. The shorter the distance between the seed plot and beans growing elsewhere, other things being equal, the greater is the chance that the beans in the seed plot will become inoculated.

3. Location of the seed plot and the bean field on soil on which beans were not produced the preceding year.

4. Cultivation of the seed plot before the cultivation of other beans when the same tool is used for both; or, better, reservation for the seed plot of a cultivator not used in other bean fields.

5. The precaution of not visiting the seed plot when vines or soil are wet. This precaution should apply to all bean fields. Care should be taken, even when the vines are dry, not to go directly into the seed plot from other bean fields.

6. Prompt removal and destruction of any plants in the seed plot found to be affected with anthracnose.

7. Application of bordeaux mixture to the plants in the seed plot several times during the season, in order to prevent infection from spores that may be carried there.

8. Selection, before the beans are harvested, of clean and otherwise desirable pods from the seed plot or other fields, in order to obtain healthy seed for planting the succeeding year's seed plot.

9. Careful harvesting of the bean plants in the seed plot and their storage in a place separate from other bean vines.

10. The threshing of the beans with a flail or in other ways so that a mixture with other bean seed cannot take place.

11. Storage of the seed in a cool, dry place until planting time the succeeding year.

12. Careful hand-picking of this seed for the increase plot or the main field in order to remove all discolored or otherwise unsatisfactory seed.

This method of controlling anthracnose in the main field will cost less than spraying five times, and is more certain of success. Much of the procedure costs nothing, but merely requires not doing certain things. The labor involved in growing a 40-acre field of beans by this method, above that ordinarily required for growing such a field, consists in selecting sufficient seed for a seed plot, in spraying the vines in this plot, in removing affected plants from it when present, in the separate harvesting and threshing of the crop, and in the careful hand-picking of the seed from it. Other work done in connection with the seed plot would have been required had it been a part of the main field.

If home-grown seed is as good as or superior to that purchased elsewhere, then growers of a large acreage of beans who have felt the loss from anthracnose, or seed dealers who are desirous of obtaining the best possible seed for their territory, should give consideration to this method. It is not to be expected that many farmers will adopt such a method, because of the detail required in order to insure success. It should be pointed out here that this method cannot be depended upon absolutely to control bacterial blight. Burkholder (1921:67) states that as a result of systemic infection of the bean plant the seeds may become infected without the pods around them showing any evidence of blight.

Procuring seed from localities where anthracnose does not occur

Observations were made for two seasons (1908 and 1909) of fields planted with Colorado-grown and eastern-grown Refugee Wax bean seed. There was no anthracnose but considerable blight present in the field planted with the western seed, while anthracnose was present to some extent in the field planted with the eastern seed. The seed procured from Colorado the second year was dry and split easily, and besides it germinated poorly, and so the yield was lower than that from the eastern seed. Edgerton (1910:50) has planted Colorado seed in Louisiana and has found it freer from anthracnose than most other seed, though much more severely affected with bacterial blight. Muncie (1917:41) reports trials in Michigan of seed grown for one year in the States of Washington, Idaho, and California. There was a little anthracnose in the seed returned from Washington but none in that from the other States. Idaho-grown seed gave very satisfactory results the first year but poor results the second year, largely because the seed was immature due to an early frost.

California-grown Early Wonder seed gave excellent results, and the plants ripened two weeks earlier in Michigan than did plants of the same variety from seed grown in Idaho and from seed grown in Michigan. Muncie believes that carefully selected seed sent to southern California to be grown for one year for seed purposes, will furnish a supply of seed year after year that will be superior to Michigan-grown seed.

Edgerton (1910:28–37) states that at the high summer temperatures in Louisiana, bean anthracnose does not develop even when spotted seed is planted; and later he suggests (Edgerton and Moreland, 1913:17) that Louisiana truckers grow their own bean seed during the summer as a second crop and thus secure seed freer from disease than that purchased from the North.

The question whether eastern growers would have better success in buying western seed than by the use of eastern-grown seed is still unanswered. It must be considered from other standpoints as well as from that of controlling anthracnose. It is true that this disease does not occur as commonly or cause as much damage in the West as in the East. It was, however, rather prevalent in Colorado in 1916 and 1917, and to an extent sufficient to cause some spotting of the seed. Bacterial blight occurs rather commonly in the West, so that one cannot depend on western seed being free from this disease. Nevertheless, eastern seedsmen have found that they can depend upon getting good seed from the West oftener than from the East, and the bean-seed-growing industry has developed rapidly in Colorado and Idaho in recent years. Most of the seed beans grown are garden and canning varieties, which command a much higher price in the East than the ordinary field sorts.

Avoiding conditions favorable for the dissemination and growth of the fungus

If it becomes necessary to plant seed having a small percentage of anthracnose infection, or if the disease appears in the field from an outside source of inoculum, or even when clean seed is planted, it is advisable to avoid as far as is practicable those conditions which favor the spread of the pathogene. Healthy plants have been grown from clean seed in experimental plots in the same field with plants that have become badly diseased from artificial inoculation with the anthracnose fungus. It has been observed also in a large number of instances that plants in hills next to inoculated plants often did not become infected although the weather was favorable for infection to take place. The disease did not spread because certain conditions favorable for it were not allowed to exist. Some of the precautions to be taken are considered in the following pages.

Manner of planting

Beans planted so close together that they form a dense mat of vines favor the spread of the disease. The vines obstruct cultivation and do not allow either the wind or the sunshine to dry out the moisture beneath them. Spores of the pathogene, if present, are transferred from one plant to others in contact, and are able under the moist conditions to germinate and penetrate the tissues of the host without difficulty.

The different varieties vary so much in their habit of growth and in the size of their seed that it is impossible to give a general recommendation for planting. Halsted (Halsted and Kelsey, 1895:28, and Halsted, 1897: 290) planted seed from 3 to 24 inches apart in rows 20 inches apart. There was a considerable decline in the proportion of spotted pods and also in the total yield of pods as the distance between the plants was increased. Halsey concludes that 4½ inches is the most satisfactory distance for Golden Wax beans. Ferris (1909:8) recommends, in addition to selecting clean seed, planting the beans in bunches in the row far enough apart so that the clusters do not touch one another. Edgerton (1909:5) finds three beans to the hill, and the hills 18 inches apart, a very satisfactory planting distance for beans grown in Louisiana. Fischer (1919) says that Riehm suggests planting in hills 50 by 50 centimeters apart instead of in drills, in order to permit good air drainage. Von Diakonoff (1909) suggests planting the rows parallel to the direction of the prevailing winds in order that the plants may be well aërated.

Anthracnose will not spread through a field of beans planted in hills as rapidly as it will through one planted in drills, because the vines in one hill are somewhat isolated from vines in other hills for a considerable part of the season, whereas in drills there is a continuous row of plants in contact with one another, and anthracnose starting at any point in the row would, under favorable conditions, spread the entire length. To secure maximum protection by hill planting, the hills should be far enough apart each way so that the vines of one hill will not come in contact with vines of other hills; and this distance will vary with different varieties. Of course the yield in such a case will be reduced below that obtained when a greater amount of seed is used, but not proportionately. It is impracticable, however, to plant hills at a distance sufficient to keep the vines of one hill from touching those of others during the latter part of the season. Just what distance apart the hills of any variety should be planted in order to secure a satisfactory yield and protection from infection has not been determined. Whatever has been said on this subject should be considered also in the light of present knowledge concerning the possibility of spore dissemination by spattering.

Good land drainage

The relation of drainage to bean anthracnose is obvious. Poorly drained soil is cold, and bean seed planted in such soil starts slowly or not at all. If the seed is diseased, the fungus develops rapidly and usually destroys the seedling before it appears above ground. During a wet season poorly drained soil tends to produce a moist condition about the base of the plants, which should be avoided. Galloway (1889) notes that the disease is worse on moist soil. Cobb (1894:383) and Querner (1908), in recommending control measures for the disease, suggest draining the land, and others suggest not choosing low, damp ground.

Removal of affected seedlings

Affected seedlings serve as a source of infection to healthy plants in the field and the garden. An early removal of them would aid in lessening the amount of disease should favorable weather for infection follow. The practice of removing affected plants was early recommended. Berkeley (1880) suggests that diseased plants be pulled and burned, and many other writers on the subject have recommended this. Whetzel (1908:433), however, has pointed out that this method is absolutely impracticable except in short garden rows. It is, of course, out of the question to attempt to remove affected seedlings from a field. However, in the seed plot and in the garden it is worth while to remove and destroy such plants, as is explained later. If the weather remains dry following the appearance of the seedling, the diseased cotyledons will drop off before further infection of the seedling takes place, as already noted (page 161). In such a case the field would be as free from disease as though every diseased seedling had been removed. Appel (1916) has observed that hilling young plants tends to prevent the spread of the disease by covering the lesions at the base of the stem, thus hindering the spores from escaping. The small lesions heal afterward, and, while the larger ones may develop and rot the plant, the spores produced are not able to be disseminated. Edgerton (1909:5) suggests that danger of the disease's spreading from affected seedlings may be lessened if the field is not entered until the cotyledons have dropped off.

Leaving the plants untouched while wet

As has been noted (page 176), a diseased plant may exist in a field of beans without the disease spreading materially to other plants. If, however, when the plants are wet, some agent passing through the field comes in contact with the diseased vine, spores are likely to be distributed to plants beyond. A very active agent in distributing spores is a horse and cultivator, or, even more so, a person picking beans. Growers of a large acreage like to start work in the fields early, and yet there are many mornings during the growing season when the dew remains on the plants until after nine o'clock. It is very important that operations in the bean field should be postponed until the vines are dry. The observance of this simple precaution will do much toward preventing a general infection of the field.

Destroying diseased vines in the fall

Since the fungus lives over winter on the old pods and vines, these may serve as a source of infection to young beans growing near them the following spring. Galloway (1889) recommends the burning of all diseased vines and pods. Both Harvey (1894) and Cobb (1894:383) believe it advisable to remove and destroy all diseased vines after harvest if the same land is to be used for beans the following year. Whetzel (1906:209) states that diseased pods and stalks should be removed from the field. The old vines and pods are not so often a source of inoculum as are the affected seeds, and yet it is unwise to disregard them altogether. One should not go to a considerable expense to remove and destroy old affected vines from the field, but should prevent their coming into contact with the new crop as far as it is practicable to do so. A rotation of the bean crop, and the application of manure containing bean refuse to other than the current or the following year's bean field, are helpful measures.

Planting at a time of the year when anthracnose is least likely to be present

Corbett (1907) suggests late planting as a possible means of avoiding the disease. This may do very well where the growing season is long, but in New York State the varieties usually planted require practically all the growing season to properly mature their pods. Since the months of July and August are drier on the average than the other months of the growing season, early-maturing varieties planted at the beginning of July might be less likely to be affected with anthracnose than if planted earlier, as the disease would not have as good an opportunity to develop from affected seeds. There are so many other factors of importance in deciding the time of planting, however, that this one of preventing anthracnose can be given little consideration.

Planting early-maturing varieties

There are years when anthracnose does not become prevalent until the fall rains come on, so that an early-maturing variety would escape infection. A variety of white pea bean known as Early Wonder is recommended by Muncie (1916) to Michigan growers because it ripens from ten to fourteen days earlier than other varieties of this kind, and thus matures before diseases have made serious invasion into the tissue of the pod, with consequent spotting of seed. This bean does not ripen appreciably earlier in New York than does the ordinary Navy Pea.

Spraying

It is interesting to note that even before the discovery of bordeaux mixture, the application of a fungicide to bean pods for the control of anthracnose had been made. Frank (1883 b:523) found that flowers of sulfur greatly decreases the number of cases of infection but provides no real security against the disease. Scribner (1888:364) states that under some circumstances applications of solutions of sulfate of iron or of copper may be made, but their use on the fruit cannot be generally recommended because of their poisonous character. He suggests the use of liver of sulfur, one ounce to five gallons of water. Galloway (1889) recommends a very early application of hyposulfite of soda, one ounce to one gallon of water, or of potassium sulfide, one-fourth ounce to one gallon of water. Two subsequent sprayings should be made after the pods are from one-half to two-thirds developed.

Five years after the introduction of bordeaux mixture into the United States, several investigators recommended its use for the control of bean anthracnose. Beach (1892:314–317, 326) experimented with cupric borate, cupric polysulfide, and bordeaux mixture. The last-named was much superior to the others and increased the yield to nearly double that of unsprayed plants. Beach emphasized the importance of spraying thoroughly and of using a nozzle that would produce a misty spray. He believed that the addition to the mixture of enough soap to make suds would cause the mixture to form a thin film over the leaves instead of minute drops. McCarthy (1892:14) recommends either an iron sulfate or a potassium sulfide solution with lime, to which molasses or glue is added as a sticker. He recommends also, as a substitute for the spray solutions, a dust mixture composed either of sulfur flour or precipitated copper carbonate diluted with some dry powdered material, as air-slaked lime or wheat flour. He believes that the application should be made as soon as the flowers have withered if there is any disease in the neighborhood. Halsted (1895 [Halsted and Kelsey] to 1901) carried on spraying experiments with beans for seven years. He used a large number of fungicides, including lime-bordeaux of various strengths, soda-bordeaux, potash-bordeaux, ammoniacal copper carbonate, eau céleste, creolin, and potassium sulfide. Soap also was used in combination with copper sulfate and with eau céleste. Halsted found that there was little difference between the regular bordeaux and the soda-bordeaux in their effects, but the other fungicides proved inferior and creolin was practically worthless. When anthracnose and blight were prevalent they were reduced materially, in most cases, by spraying with bordeaux. With the two successive crops of 1896 (Halsted, 1897:330) and the two of 1897 (Halsted, 1898:310, 312), the check row yielded more beans than did the sprayed rows. During the dry seasons of the year 1900 (Halsted, 1901 b:420, 421), little or no success was obtained from spraying. Whetzel (1906:208) relates an instance in which a single spraying reduced the amount of anthracnose 20 per cent. Blin (1906) obtained relatively good results in France during 1901 and 1902 from spraying with bordeaux. Von Diakonoff (1909) advises bordeaux or a copper-sugar-lime mixture.

County agricultural agents have occasionally reported instances in which spraying has given good results in preventing the disease. Nearly all the spray calendars and bulletins on the subject issued by the various experiment stations include spraying with bordeaux mixture in their recommendations for control of bean anthracnose. In later years, instances have occurred in which bordeaux was reported as not successful in controlling the disease although the vines were well sprayed.

Fairchild (1894), White (1906:95), Corbett (1907), and Whetzel (1908: 433) question the practicability of spraying beans generally, although granting that there may be circumstances under which it could be recommended. Whetzel has succeeded, however, in controlling the disease on a few rows by thoroughly and properly applying the mixture with a hand machine. He says that the field machines are not constructed so that the mixture can be applied as thoroughly and effectively as by hand. He believes that until a machine is put on the market capable of delivering a spray that will cover the stems and the pods at any stage in the growth of the plant, little or no results can be expected from spraying, and even then the practice will be very expensive because of the machinery, chemicals, and labor involved. Hollman (1915) sprayed plants grown from affected seed with 1-per-cent bordeaux mixture without much success, but he advises its use as a protective measure. Fischer (1919:249) tells of Schander and Krause's experiments at Bromberg, where a 1- or 2-per-cent soda-bordeaux and a 1- or 2-per-cent lime-bordeaux were used. The soda-bordeaux killed the plants and the lime-bordeaux injured them, at least when the solution contained just enough lime to neutralize the copper sulfate solution. Later experiments with 1-per-cent bordeaux, even when seed from healthy pods sorted from diseased plants was used, reduced only slightly the number of affected pods and did not prevent the disease from appearing on the leaves and stems.

The writer conducted spraying experiments during the years 1908 and 1909 in large fields of canning beans at Oneida, New York, in cooperation with the Burt Olney Canning Company, in small experimental plots at Oneida during 1908, 1909, and 1910, and at Ithaca, New York, for six years after 1910. The field sprayings were conducted on 200 acres or more of beans. These were sprayed five times during the growing season, at intervals of about ten days, the applications beginning when the plants were just out of the ground and continuing until after picking had begun. Bordeaux mixture (4–4–50) was used on part of each field and lime-sulfur (1–50) on another part, the two sprayed areas being separated by fifteen unsprayed rows. With the varieties of beans grown, Refugee Wax and

Refugee Green Pod, the pods and the undersides of the leaves, which are the particular points of attack, are so protected by their bushy growth that it is very difficult to apply the mixture thoroughly to these places with the ordinary field sprayer. A Niagara gas sprayer was used and the mixture was delivered at 100 pounds pressure from near the ground to the sides of the plant (fig. 20). Two and sometimes three machines were kept busy during the spraying season. In 1908 the company expended $1000 for bean spraying alone, and in 1909 the expenditure was $1250. Each application cost about $1 an acre. Thus the spraying question was of sufficient importance to the officials of the company for them to wish to have its value demonstrated, and this in spite of the fact that they had been spraying for the past seven years.

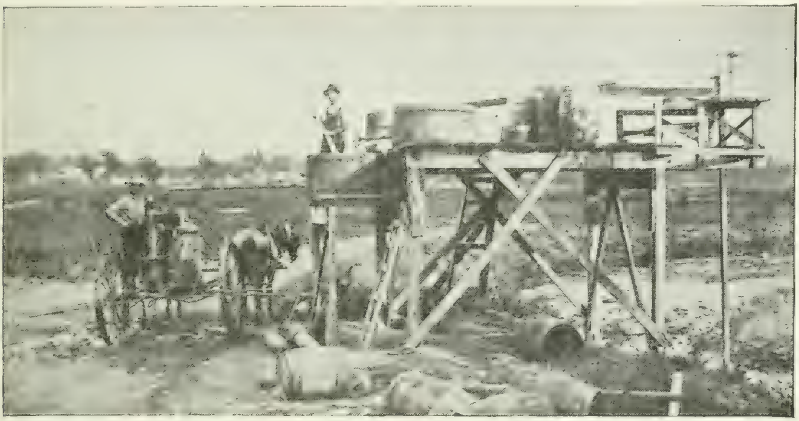

FIG. 20. PLATFORM AND APPARATUS FOR PREPARING BORDEAUX MIXTURE IN LARGE QUANTITIES, AND SPRAYING MACHINE DEVISED ESPECIALLY FOR SPRAYING BEANS

A calculation of the yield, and of the amount of anthracnose and blight, for the three center unsprayed check rows and for the three bordeaux-sprayed rows adjoining, was made at each picking of every field. A calculation of the yield of plants sprayed with lime-sulfur was made from only a few fields.

The amount of anthracnose and of blight during the two years was negligible. The difference in yield between sprayed and unsprayed rows was slight, being one year in favor of the sprayed rows and the following year in favor of the unsprayed, and in both cases being well within the range of experimental error. The company has since discontinued spraying beans.

During the eight years since 1908 a few rows of beans have been thoroughly sprayed with bordeaux, and a few with lime-sulfur, a hand machine being used. From four to six thorough applications were made throughout the season, beginning soon after the plants appeared and continuing until the pods were about two-thirds grown. The first year neither anthracnose nor blight appeared to any appreciable extent, even on the check rows. In 1909 no anthracnose was present, but the blight appeared to be controlled somewhat by the application of the spray. With one variety, Refugee Green Pod, there was no blight on the sprayed rows while in the unsprayed rows 3 per cent of the pods were spotted with blight. On the Red Marrows that were sprayed 2 per cent of the pods were spotted with blight, while the unsprayed rows had 11 per cent of spotted pods.

In 1910 both anthracnose and blight were again negligible. In 1911, one-third of each of six rows was planted on June 22 to seed of several varieties mixed together, all badly spotted with anthracnose. Mixed varieties were used because it was not possible to obtain a sufficient quantity of affected seed of one variety. The remaining two-thirds of the six rows were planted at the same time to seed selected from clean pods, three rows to Red Marrow and three to Navy Pea. The first and fourth rows were sprayed with bordeaux, the second and fifth rows were not sprayed, and the third and sixth rows were sprayed with lime-sulfur. Powdered arsenate of lead at the rate of 2 pounds to 50 gallons was added to both the bordeaux and the lime-sulfur, in the hope of killing insects that might be instrumental in carrying the blight organism. Five applications were made during the season, on June 13, July 1, 14, and 25, and August 9. One-half of the plants in that part of the plot farthest from the end planted to spotted seed were inoculated with a suspension of spores of two strains of *Colletotrichum lindemuthianum* which had proved virulent on the two varieties. This left one-half of the plants from clean seed as a middle third of each row uninoculated with the fungus in any way except with spores that might be carried to them. Two inoculations were made, one on July 17 when one-half of the plants set aside for this purpose were inoculated, and the other on August 22, when the remainder were inoculated. These inoculations were made at times when the weather promised exceptionally favorable conditions for infection, and it proved to be so in each case. The plan of the experiment may be outlined as follows:

Applications	Clean seed		Spotted seed, plants not inoculated artifically
	Plants inoculated artificially	Plants not inoculated artificially	
Bordeaux I	Red Marrow	Red Marrow	Mixed varieties
Not sprayed I	Red Marrow	Red Marrow	Mixed varieties
Lime-sulfur I	Red Marrow	Red Marrow	Mixed varieties
Bordeaux II	Navy Pea	Navy Pea	Mixed varieties
Not sprayed II	Navy Pea	Navy Pea	Mixed varieties
Lime-sulfur II	Navy Pea	Navy Pea	Mixed varieties

The spotted seed was largely of the wax type, and the pods were picked when a majority were of marketable size as string beans. The results from this experiment are given in table 7. The healthy pods, after being sorted out, were left in a basket for three days. They were then sorted again and the percentage of pods that had spotted in the meantime was

TABLE 7. YIELD, AND PERCENTAGE OF PODS SPOTTED WITH ANTHRACNOSE AND BLIGHT, FROM SPRAYED AND FROM UNSPRAYED ROWS OF SNAP BEANS GROWN FROM SEED BADLY SPOTTED WITH ANTHRACNOSE, IN 1911

Treatment	Date of picking	Number of plants in row	Number of healthy pods	Pods spotted with anthracnose (per cent)	Pods spotted with blight (per cent)	Additional pods spotted with anthracnose after being picked three days (per cent)
Bordeaux I	August 13	62	307	0.6	1.2	0.3
Check I	August 13	71	663	1.7	6.7	20.0
Lime-sulfur I	August 13	62	380	1.8	12.8	0.0
Bordeaux II	August 17	71	722	4.0	0.1	3.3
Check II	August 17	70	557	18.8	4.5	10.7
Lime-sulfur II	August 17	82	592	2.0	1.5	0.5

determined. No importance is attributed to the comparative yield in number of pods given in table 7, as the seed was mixed and more plants of a prolific nature may have been present in one row than in another.

It is seen from table 7 that there was a decided control of the disease by spraying with either bordeaux or lime-sulfur. The control is especially noticeable on pods three days after picking.

The results with field beans grown from healthy seed are given in table 8. It is seen from this table that spraying the vines with either bordeaux or lime-sulfur materially reduced the percentage of spotted pods due to anthracnose. The spraying does not appear to have controlled the blight.

The pods sprayed with bordeaux in the above experiments were decidedly discolored by the precipitate from the mixture. Fawcett (1907) points out that this may be avoided by making the last application with ammoniacal copper carbonate, which leaves no stain. The pods sprayed with lime-sulfur showed no evidence of stain at picking time.

In 1912 complete results from spraying were obtained from only a small plot, two-thirds of which consisted of Red Marrows planted with seed from clean pods while the other third of each row was planted with spotted seed of mixed varieties. The planting was done on June 13, and applications of fungicides were made on July 7, 17, and 26, and August 6. Bordeaux (4–4–50) and lime-sulfur (32° Baumé, 1–50) were the fungicides used. The results are given in table 9. From this table it is seen that no control of anthracnose was obtained from spraying, and that seeds from clean pods were nearly as badly affected as those from spotted seed. Earlier in the season (July 26), however, the plants from clean seed had shown practically no infection, but infection was common on the plants from spotted seed. Cool, rainy weather during August brought about abundant infection from numerous sources.

In 1913 spraying was done on seven varieties planted in eleven plots 50 feet long. Plots 1, 2, and 3 were planted with seed a small percentage of which was spotted with anthracnose; plots 4, 5, 6, 7, 8, and 9 with seed a large percentage of which was similarly spotted; and plots 10 and 11 with seed from selected clean pods. The planting was done on June 29. The Red Valentine and Challenge Black Wax beans did not germinate well and were replanted on July 12. Applications of fungicides were made on July 16 and 26, and August 6 and 16. The Navy Pea and Red Kidney

TABLE 8. YIELD, AND PERCENTAGE OF PODS SPOTTED WITH ANTHRACNOSE AND BLIGHT, FROM SPRAYED AND FROM UNSPRAYED ROWS OF FIELD BEANS GROWN FROM SELECTED HEALTHY SEED, IN 1911

	Variety	Treatment	Number of plants	Yield of dry pods (grams)	Yield of seed (grams)	Pods spotted with anthracnose (per cent)	Pods spotted with blight (per cent)	Clean pods (per cent)	Spotted seed (per cent)
Not inoculated artificially	Red Marrow	Bordeaux.....	52	1,255	806	25.0	6.5	68.5	0.0
		Check........	58	1,335	947	76.0	0.0	24.0	18.0
		Lime-sulfur...	56	1,045	765	20.0	3.5	76.5	10.0
	Navy Pea	Bordeaux.....	49	936	704	0.0	5.0	95.0	0.0
		Check........	47	935	715	36.0	11.0	53.0	10.0
		Lime-sulfur...	48	1,115	850	12.0	14.0	74.0	1.0
Inoculated artificially twice during season	Red Marrow	Bordeaux.....	114	2,525	1,830	33.0	5.3	61.4	0.0
		Check........	118	2,055	1,455	82.0	1.0	17.0	21.0
		Lime-sulfur...	118	2,010	1,452	27.5	5.5	67.0	17.0
	Navy Pea	Bordeaux.....	92	1,575	1,220	4.0	5.5	90.5	1.0
		Check........	90	1,610	1,215	49.0	4.5	46.5	10.0
		Lime-sulfur...	92	1,675	1,275	24.0	5.0	71.0	3.5

beans, since they would mature later, were given another application on September 8. Bordeaux (5-5-50), Pyrox (1 pound to 5 gallons of water, as recommended on the label), and lime-sulfur (32° Baumé, 1–50), were the fungicides used. A killing frost occurred on September 15. Only the percentage of pods spotted with anthracnose is given in table 10, showing the results:

TABLE 9. YIELD, AND NUMBER AND PERCENTAGE OF RED MARROW BEAN PODS SPOTTED WITH ANTHRACNOSE, FROM SPRAYED AND FROM UNSPRAYED ROWS IN 1912

Seed planted	Treatment	Number of pods	Pods affected with anthracnose	
			Number	Per cent
Clean seed	Bordeaux	2,600	2,450	93
	Check	1,452	1,450	99
	Lime-sulfur	1,983	1,950	98
Spotted seed	Bordeaux	392	392	100
	Check	227	227	100
	Lime-sulfur	295	295	100

TABLE 10. PERCENTAGE OF PODS SPOTTED WITH ANTHRACNOSE IN SPRAYED AND IN UNSPRAYED ROWS, FROM ELEVEN PLOTS CONTAINING SEVEN VARIETIES OF BEANS, IN 1913

Plot	Variety	Treatment			
		Bordeaux	Pyrox	Not sprayed	Lime-sulfur
1	Red Kidney	0.0	0.0	0.0	0.0
2	Crystal Wax	*2.4	*0.2	*1.4	*1.0
3	Red Valentine	0.0	0.6	0.0	0.0
4	Challenge Black Wax	0.2	0.8	57.2	0.8
5	Navy Pea	0.4	0.4	0.4	0.4
6	Red Kidney	4.6	6.2	58.6	17.4
7	Crystal Wax	0.2	7.0	36.4	20.8
8	Golden Wax	0.0	0.0	0.0	0.0
9	Refugee Wax	0.0	0.0	0.0	0.0
10	Red Kidney	0.0	0.0	0.0	0.0
11	Navy Pea	0.0	0.0	0.0	0.0

* The cotyledons showed anthracnose lesions.

The season was dry and anthracnose was not abundant, but the results on plots 4, 6, and 7, the only ones having a considerable amount of anthracnose in the checks, lead to the conclusion that the fungicides were effective in the following order: bordeaux, Pyrox, lime-sulfur, with the latter falling short somewhat.

Spraying was continued during 1914 on Refugee Wax, Davis Wax, Flageolet Wax, an unidentified wax, and Burpee's Stringless Green Pod. Rows 50 feet long of each variety were planted on June 2, on plots situated side by side on the same land that was used for this purpose in 1913 (table 11). The Flageolet Wax contained a large percentage of seed spotted with anthracnose, but the seed of the other varieties included only a small amount of spotted seed. The row of Flageolet Wax had many missing hills. Applications of the fungicides were made on July 2, 15, and 26, and August 9. One row of each variety was sprayed with bordeaux (5-5-50), one row of each, except the unidentified wax, with Pyrox (1 pound to 5 gallons of water), and one row of each except Flageolet Wax and the unidentified wax with Sulfocide, a commercial sulfur solution (1 gallon to 200 gallons of water). The remaining row of each variety was left unsprayed as a check.

In order to insure anthracnose infection, two inoculations with spores of *Colletotrichum lindemuthianum* obtained from cultures were made, one on August 2 and the other on August 16. Favorable weather for infection followed in each case, and the weather at Ithaca during the summer continued favorable for the development of the pathogene. All the varieties matured their pods before frost and a record of healthy and spotted pods was made on October 8 (tables 11 and 12).

This experiment shows that all the spray materials used had value in the control of anthracnose and blight. The plants from spotted seed were badly affected, and the control of the disease by spraying was not so good on them as on plants from clean seed.

In 1915 but one variety, Davis Wax, was used in the spraying experiment. Two rows 50 feet long were planted on June 8, one to be sprayed and one to be left as a check. One-third of each row was planted with seed of this variety spotted with anthracnose, and the other two-thirds with seed from selected clean pods. A thorough natural inoculation took place, and so it was not necessary to inoculate artificially. The beans appeared on June 16 and applications of a fungicide were made on June 19, July 3, 10, 24, and 30, and August 7. Pyrox (1 pound to 5 gallons of water) was used for the first three applications and bordeaux 5–5–50

TABLE 11. YIELD, AND NUMBER AND PERCENTAGE OF PODS SPOTTED WITH ANTHRACNOSE AND WITH BLIGHT, IN SPRAYED AND IN UNSPRAYED ROWS IN 1914

Variety	Treatment	Total number of pods	Pods spotted with anthracnose		Pods spotted with blight	
			Number	Per cent	Number	Per cent
Refugee Wax	Bordeaux	1,120	3	0.3	5	0.5
	Pyrox	1,134	10	0.9	3	0.3
	Check	1,467	129	8.8	7	0.5
	Sulfocide	1,307	93	7.1	4	3.1
Davis Wax	Bordeaux	612	33	5.4	12	2.0
	Pyrox	475	19	4.0	11	2.3
	Check	344	23	6.6	59	17.2
	Sulfocide	173	10	5.8	27	15.6
Flageolet Wax	Bordeaux	74	6	8.0	5	6.8
	Pyrox	90	23	25.8	3	3.3
	Check	174	151	86.8	4	2.3
Unidentified wax	Bordeaux	506	20	4.0	5	1.0
	Check	252	46	18.3	13	5.2
Burpee's Stringless Green Pod	Bordeaux	776	76	9.8	10	1.3
	Pyrox	670	40	6.0	9	1.3
	Check	641	131	20.3	28	4.3
	Sulfocide	411	55	13.4	27	6.6

TABLE 12. AVERAGE PERCENTAGE OF PODS SPOTTED WITH ANTHRACNOSE AND WITH BLIGHT FROM THE VARIOUS SPRAYED AND UNSPRAYED PLOTS ON THE RESPECTIVE VARIETIES EMPLOYED, IN 1914

Treatment	Anthracnose (per cent)	Blight (per cent)
Bordeaux	5.5	2.3
Pyrox	9.2	1.8
Check	28.2	5.9
Sulfocide	8.8	5.1

for the other three, the change to bordeaux being made because of the injury resulting to the vines from the use of Pyrox; three hills, in fact, were killed, and the others on July 15 looked very straggling from the loss of their lower leaves, so that they presented a much less satisfactory appearance than the unsprayed rows, even though they were but little affected with anthracnose. The results of this experiment are given in table 13:

TABLE 13. YIELD OF DAVIS WAX BEANS, NUMBER AND PERCENTAGE OF PODS AFFECTED WITH ANTHRACNOSE, AND WEIGHT OF GOOD AND OF CULL SEEDS, FROM SPRAYED AND FROM UNSPRAYED ROWS IN 1915

Seed planted		Sprayed	Not sprayed
Spotted seed	Number of plants	26	30
	Number of pods	122	129
	Number of pods affected with anthracnose	43	129
	Per cent of pods affected	35	100
	Weight of good seed (grams)	79	10
	Weight of cull seed (grams)	17	52
Clean seed	Number of plants	66	86
	Number of pods	240	521
	Number of pods affected with anthracnose	22	521
	Per cent of pods affected	9	100
	Weight of good seed (grams)	290	89
	Weight of cull seed (grams)	49	410
Total	Number of plants	92	116
	Number of pods	362	650
	Number of pods affected with anthracnose	65	650
	Per cent of pods affected	18	100
	Weight of good seed (grams)	369	99
	Weight of cull seed (grams)	66	462

This experiment, although not conducted on an extended scale, shows conclusively the control that can be obtained by spraying the plants thoroughly with bordeaux mixture a sufficient number of times during the season to protect them.

Spraying was continued in 1916 on Davis Wax and Refugee Wax beans. Two rows 40 feet long of each variety were planted on June 10; spotted seed was used in one-third of each row, and healthy seed in the remainder. No artificial inoculations were made. One row of each variety was left unsprayed as a check, and one of each was sprayed with bordeaux mixture (5-5-50) on July 3, 12, and 24, and August 3, 14, and 24. Data obtained at harvest, October 4, are given in table 14:

TABLE 14. YIELD OF DAVIS WAX AND REFUGEE WAX BEANS, AND NUMBER AND PERCENTAGE OF PODS AFFECTED WITH ANTHRACNOSE, FROM SPRAYED AND FROM UNSPRAYED ROWS IN 1916

Variety	Treatment	Number of plants	Total number of pods	Pods affected with anthracnose		Weight of good seed (grams)	Weight of cull seed (grams)
				Number	Per cent		
Davis Wax	Sprayed......	59	520	93	18	652	64
	Not sprayed..	61	437	184	42	175	93
Refugee Wax	Sprayed......	80	770	16	2	370	104
	Not sprayed..	74	662	121	18	......	....

It seems to the writer, in view of the results of these experiments, that anthracnose and possibly blight may be kept in check by thoroughly spraying the plants with bordeaux mixture, if the operation is begun soon after the plants have appeared above ground and continued at intervals of about ten days until the pods are reaching marketable size. The nature of the growth of the plants determines to a considerable degree whether they may be sprayed well with a spraying machine. Varieties such as those used in the field experiments at Oneida are so bushy that it is very difficult to spray them thoroughly, while such varieties as Crystal Wax, Currie, Davis, Detroit, Double-Barrel, and others, which have a more erect growth, although compact, are less difficult of such treatment.

The field experiments at Oneida show conclusively that it does not pay to spray during a dry season. Spraying experiments conducted on a small scale with hand sprayers have shown that it is possible to control the disease to a considerable extent by this method. Whether the disease can be controlled as well with traction or power machines during epiphytotic years is yet an unsettled question.

In regions where anthracnose usually occurs annually, spraying can probably be used to advantage if the practice is supplemented by the use of clean seed. In New York spraying is not practicable for growers of dry beans, and probably not for growers of canning beans. If the total cost of making five applications is $7.50 an acre, it would be necessary for growers of dry beans to get from spraying an increase of 1.5 bushels an acre each year with beans selling at $5 a bushel in order to balance accounts. A decided increase in yield could be expected in a year when anthracnose was prevalent, but none if the season were dry. If an epiphytotic of the disease occurs in two out of seven years, as was the case from 1908 to 1915, then it would be necessary for the grower to get an increase from spraying of about 5.25 bushels in each of such two years in order to come out even. In order to make spraying pay, he must get a greater increase in yield or the selling price must be greater, and the time spent in spraying must not be at the expense of other farm operations.

Applying a fungicide to picked pods before shipping

Rolfs (1897) recommends the application of a sulfur spray just before packing pods for shipping, in order to kill the spores adhering to the pods. Fawcett (1907) also recommends the application of either potassium sulfide or a soda-sulfur spray. He says the pods should be spread out on the floor and the spraying solution thoroughly applied, the pods being turned over during the operation so that all the surfaces will be covered. The pods should be allowed to dry before being crated. In order to guard further against infection, Kirk (1905) suggests the exclusion of affected pods in packing, and Fulton (1908:12) says that affected pods should not be picked, washed, nor packed with sound ones. Containers should be so devised as to allow good ventilation, and the pods should be stored in as dry and cool places as is possible. Since there will be no anthracnose infection on pods during shipment if the disease is controlled in the field, stress should be placed on such control.

Application of a fungicide to the soil

Halsted (1900 a:380) is the only investigator who has ever recorded the application of any fungicide to the soil in an effort to control bean anthracnose, and in his experiment there was no anthracnose on any of his bean plants throughout the season although blight was present to some extent. He applied bordeaux mixture to the soil at the rate of 4320 gallons to the acre. In the first crop of the year the blight was reduced one-half on leaves and pods, but in the second crop there was just as much blight on these plants as there was on the check. Moreover, the plants growing on land that had received the application were smaller than those on the untreated soil.

Rotation of crops

The idea that the fungus is able to winter in the soil has led many writers to recommend, among other suggestions, that the grower should not plant beans on land that bore a diseased crop the previous season. Harvey (1894:153) calls attention to this, and in the same year Cobb (1894) advises a change of ground. Halsted (1900 b: 127), after growing beans each year on the same ground for a number of successive years, found that the plants yielded more in the first four years than when grown on new land, but less afterward. Usually a greater number of spotted pods were produced on plants grown year after year on the same land.

Observations were made by the writer on beans grown as the seventh successive crop on the same land. They appeared as thrifty, and yielded as well, as any crop grown on rotated land. Moreover, they were as free from anthracnose and blight as the others, although in fairness it must be said that the summers had been dry for the three years preceding the observations. There is little chance of anthracnose occurring where beans are planted year after year on the same ground, if the vines, when affected, are not returned to this soil. There is greater danger that such a practice will introduce other diseases, especially stem rots, which tend to reduce the yield materially.

Selection of resistant plants

As has been stated earlier in this paper (page 149), no variety of *Phaseolus vulgaris* has been found to be absolutely immune to anthracnose, although a selection of the ordinary Red Kidney has been obtained which has proved to be resistant to such an extent that it will produce a very satisfactory yield in years when the ordinary Red Kidney becomes badly affected, and a Nova Scotia White Marrow has been located that shows considerable resistance in the field although susceptible in the seedling stage when inoculated artificially. Further progress in obtaining resistant beans with these as a basis for hybridization is being made, the resistance being readily tested by artificial inoculation in the field, the cold frame, or the green-house. Selections can be made of large numbers of individuals by artificially inoculating them while in the seedling stage. Any showing marked resistance can be allowed to mature seed, and further tests can be made with them. Investigators are beginning to realize that the selection of plants resistant to disease is a most practical and satisfactory method of disease control, and even now work of this kind is engaging the attention of phytopathologists to a very marked degree.

As already stated (page 149), there are many varieties of beans which, while susceptible to one strain of the pathogene, are resistant to the other. It has been observed that often only one variety of beans is grown in a given locality; for example, Shepard (1919) reports that in 1918 pea beans constituted 76 per cent of all beans grown in Orleans County and 77 per cent of all grown in Monroe County, while in Yates County only 6 per cent of all beans are pea beans while 30 per cent are White Marrow, 29 per cent are Yellow Eye, and 15 per cent are Red Kidney. It happens that the pea bean referred to is susceptible to strain *alpha* of the pathogene but resistant to strain *beta*, while the other varieties mentioned are susceptible to strain *beta* but resistant to strain *alpha*. It is very likely that in regions where the pea bean is grown, strain *beta* does not exist to any great extent, and the introduction of clean seed of varieties resistant to this strain. even if susceptible to the other, would give crops of these varieties free from anthracnose for a few years. Eventually the *beta* strain would appear, and, its host being prevalent, would develop abundantly in years favorable for it. Similar results could be expected from the introduction of the pea bean, or any other variety resistant to the *beta* strain, into localities where this strain is the only one prevalent. There are other factors, to be sure, which tend to discourage a change in varieties in a given locality, and these have largely operated to prevent such changes.

Consideration of prophylactic measures

Bean anthracnose occurs to a serious extent in New York only during two or more wet seasons in succession, and the losses occasioned at such times are often forgotten by the grower in the dry seasons that intervene. It is a question with him then whether the time and the money needed for successful control measures would not be lost in carrying them out. At the time when the disease occurs, however, he is willing to do anything to lessen its destructiveness. With bean anthracnose, as with most other plant diseases, control measures to be successful must be given consideration before the disease appears, or, better, before the crop is planted; and whatever measures are decided upon must be carried out completely. Frantic attempts to control the disease after it has appeared are usually a waste of time and money and cannot be generally recommended.

When one considers the various measures suggested for the control of this disease, he must realize that each has disadvantages and that the cost would be greater than the gain were all carried out even though the disease was thereby controlled. Some measures offer a greater promise of success than others. These are here summarized and briefly discussed.

Seed disinfection may prove to be worth while, but more experimental work is necessary before it can be recommended.

The avoidance of conditions favorable to the fungus is a precautionary measure that should receive consideration from all bean growers since it involves little or no expense.

Spraying is not a practicable measure for growers of dry beans in New York, but frequent and thorough applications of bordeaux mixture can be depended on to protect the plants from anthracnose; and such a control measure is practicable when the value of the crop is sufficiently great to warrant the expense involved.

As far as present evidence indicates, the application of a fungicide to the soil is impracticable and ineffective.

Rotation of crops, in itself, will not prevent anthracnose nor reduce the liability of its occurrence to any marked extent; but it is a measure that should receive some consideration, since its practice may serve to keep down the losses occasioned by other diseases.

The disinfection of picked pods need be considered only by growers of snap beans who ship to distant markets. This will not be necessary for growers who are successful in controlling the disease in their fields.

The use of anthracnose-resistant varieties is a satisfactory means of control and should be taken advantage of when possible.

The practice of careful hand-picking in order to remove diseased seed cannot be depended upon as an effective control measure because affected seed will escape the eye of the most practiced observer. However, this practice will reduce the amount of affected seed planted, and if the seed is picked over at least twice the chances of getting a clean crop will be greatly improved as compared with the use of seed that has not been carefully hand-picked.

SUMMARY

Bean anthracnose is a disease of beans, principally affecting varieties of *Phaseolus vulgaris* L., which was first definitely reported and described in 1878 although it probably existed at a much earlier date. It has since been reported from every continent, and from nearly every country where beans are grown.

The disease has caused very large losses in years favorable for its development. In the eastern part of the United States, epiphytotics have occurred in the years 1906 to 1908 and 1914 to 1917.

All parts of the plant, even the roots, are subject to the disease, but it is most noticeable on the pods, where it forms dark, sunken cankers which subsequently extend to the seed contained within.

The spores germinate readily at room temperatures, within twenty-four hours in nutrient culture media and in bean agar and more slowly in water, the germ tube usually forming an appressorium on contact with a hard substratum.

A moderate growth of mycelium is produced in culture, which becomes dark-colored and forms acervuli from which a flesh-colored mass of spores is exuded. Under favorable conditions spores may be produced after three days growth. Cultures lose their power to produce spores by age or continued exposure to temperatures above the optimum. As the culture dries, setae are produced within and around the acervuli.

The minimum temperature for growth lies between 0° and 4° C., the optimum near 22°, and the maximum between 34° and a few degrees lower.

The fungus after entering the host extends its hyphae horizontally and diagonally into the cells. Browning of the cell contents occurs soon after the attack. The walls collapse and a lesion may be observed, at the earliest, four and one-half days after inoculation.

A stroma is formed at about the time when the cells collapse, and from it arise, in various places, a cluster of conidiophores which push up the epidermis, forming pimples. Spores are produced at the extremity of the conidiophores, and these spores break through to the surface as a pasty mass. The fungus is capable of living from one season to another, and even for two seasons, in old affected vines and pods, which may serve as a source of inoculum when carried to the field. Spores washed into the soil are not viable to any extent after seven weeks.

The fungus when it invades the seed is able to live in its tissues in a more or less inactive condition for at least two years when the conditions of seed storage are not unfavorable for it.

Plants in the seedling stage and the youngest parts of older plants are the most susceptible to the disease.

There are at least two strains or biologic forms of the fungus. Many varieties of *Phaseolus vulgaris* resistant to the one are susceptible to the other, and vice versa. There are some varieties that are very susceptible to both strains, and at least one that is highly resistant to both. The wax-podded bush beans include a larger percentage susceptible to both strains than are found in other groups of varieties.

Several other species of Phaseolus are susceptible to anthracnose, but in this country, with rare exceptions, the disease is not severe nor often noticeable on such plants.

A few species of the related genera Vigna (cowpea) and Dolichos (kulthi and val bean) have shown some susceptibility in a few cases, but plants of other genera which have been inoculated have shown complete immunity.

Varieties of *Phaseolus vulgaris* are not susceptible to infection by fungi causing anthracnose of other plants, so far as infection experiments on them have been tried.

Selection of seed from clean pods has given a crop free from anthracnose in nearly all cases, and has always given anthracnose-free seedlings in clean soil.

Western-grown bean seed gave crops free from anthracnose but with considerable blight.

Spraying with bordeaux mixture throughout the season has prevented anthracnose to a large extent even in seasons favorable for it. In dry seasons spraying does not increase the yield. Applications of lime-sulfur solution and of two prepared fungicides gave some control, but not as good control as did bordeaux mixture.

The use of resistant varieties gave greatest promise of a satisfactory means of control.

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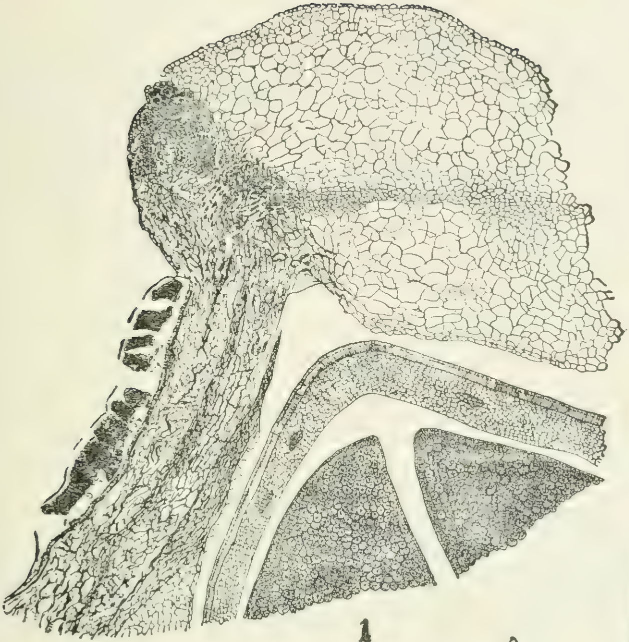
Memoir 39, *The Genetic Relations of Plant Colors in Maize*, the third preceding number in this series of publications, was mailed on July 19, 1921.

PLATE I

PATHOLOGICAL ANATOMY OF BEAN ANTHRACNOSE

1, Section across an anthracnose lesion on an immature pod, showing depth of lesion, affected tissues, and spore masses. Drawn with aid of camera lucida. × 25

2, Section across a lesion on pod of Golden Refugee bean, showing an acervulus with spores and several setae. Drawn with aid of camera lucida. × 166



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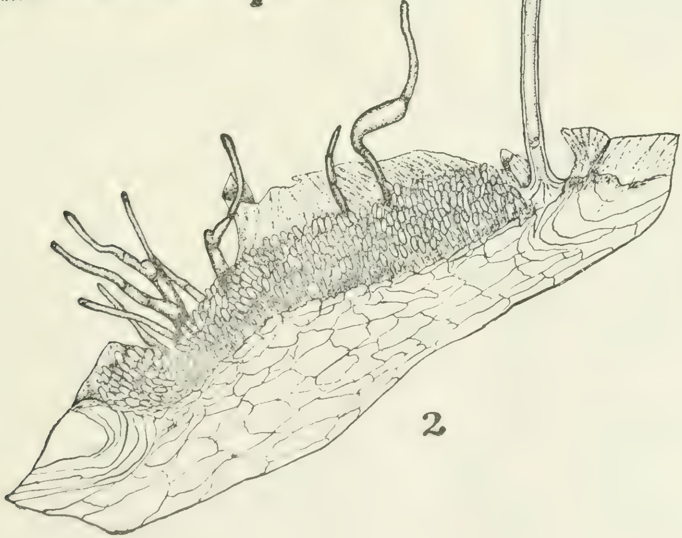

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PLATE II

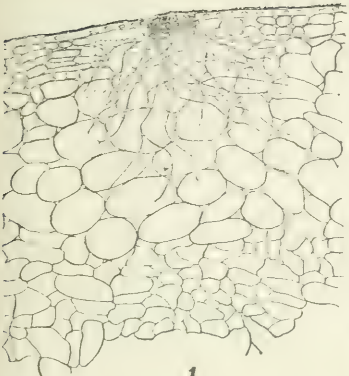
DEVELOPMENT OF COLLETOTRICHUM LINDEMUTHIANUM WITHIN HOST TISSUE

1, Section across very small, newly formed lesion on Refugee Wax bean pod. The dotted part represents discolored area as seen in the microscope, the mycelium extending beyond this. Spores are being produced in a pocket beneath the cuticle, which is not yet ruptured. × 55

2, Same tissue, higher magnification. The disorganization of subepidermal cells can be noted. × 166

3, A few cells from cross section of an infected wax-bean pod, showing advancing fungous hyphae

4, Section across recently invaded tissue of outer coat of affected pod, showing twelve fungous hyphae penetrating the wall of a cell. × 333



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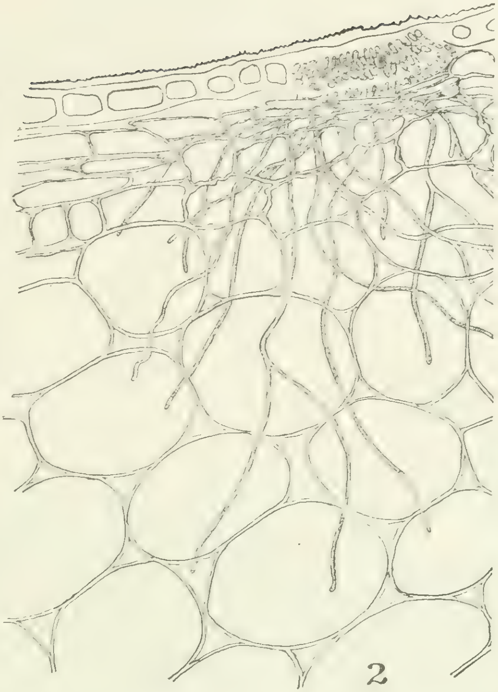

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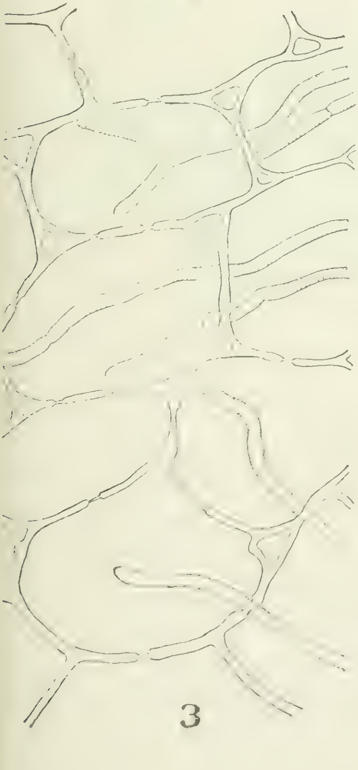

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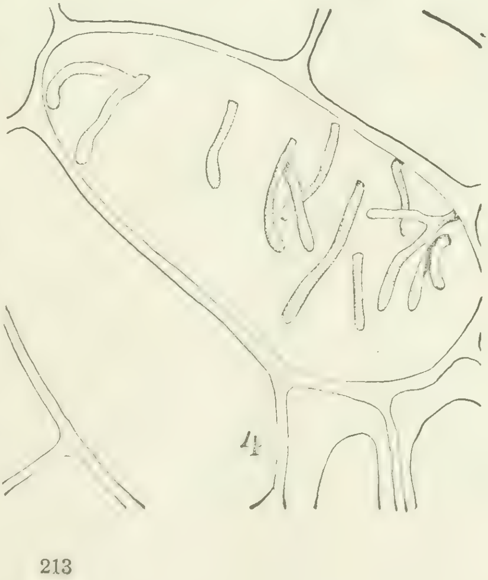

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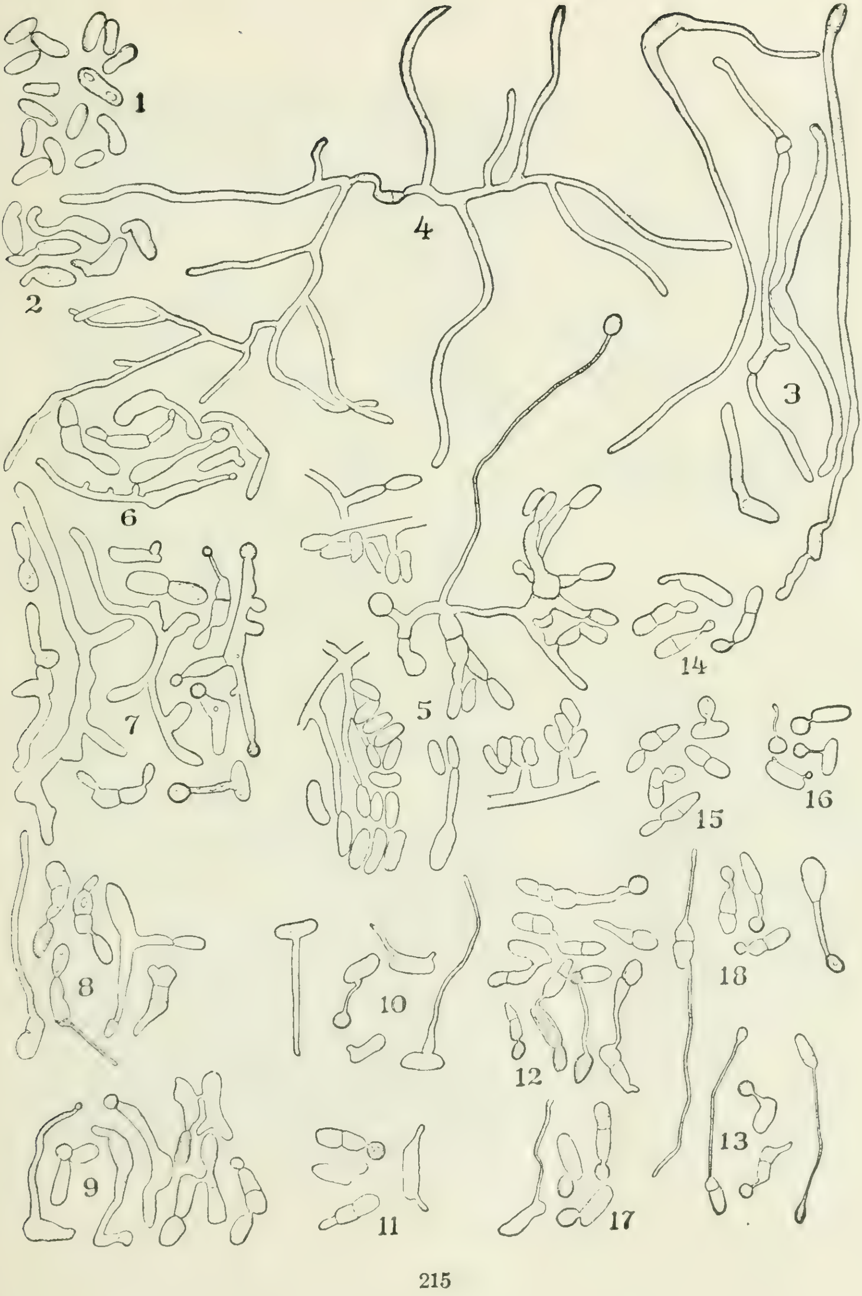
PLATE III

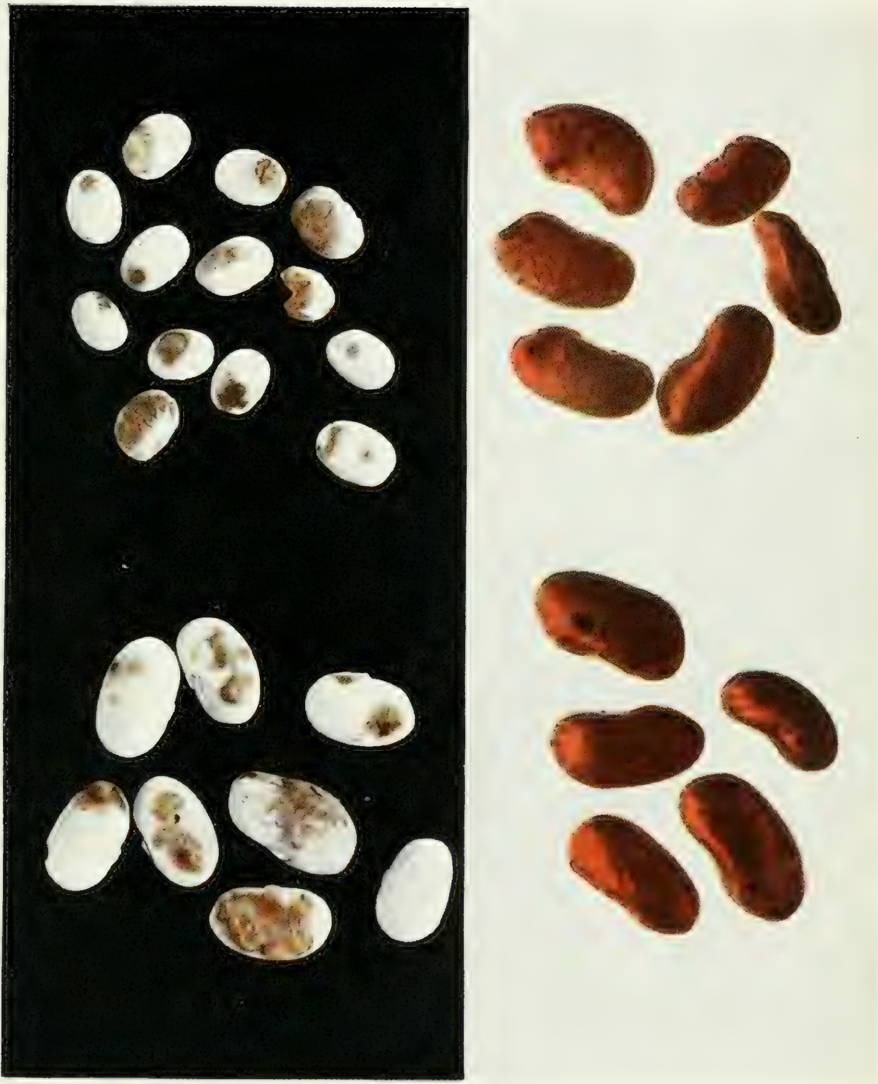
CONIDIA OF COLLETOTRICHUM LINDEMUTHIANUM

(Temperature varied from 20 to 24° C.)

1, Conidia from an acervulus, × 342. 2, Conidia germinating after 9 hours in bean agar, × 339.47. 3, Germination after 20 hours in bean agar, × 347.36. 4, Germination after 54 hours in agar, × 166.73. 5, Spore formation after growth of 4 days in agar, × 347.36. 6, Germination and production of appressoria after 19 hours in sugar solution, × 339.47. 7, After 36 hours in sugar solution, × 347.36. 8, After 66 hours in sugar solution, × 347.36. 9, After 84 hours in sugar solution, × 363.15. 10, After 18 hours in water, × 347.36. 11, After 37 hours in well water, × 363.15. 12, After 65 hours in well water, × 347.36. 13, After 9 days in well water, × 363.15. 14, After 36 hours in distilled water, × 363.15. 15, After 62 hours in distilled water, × 363.15. 16, After 8½ days in distilled water, × 363.15. 17, After 38 hours in rain water, × 363.15. 18, After 66 hours in rain water, × 347.36

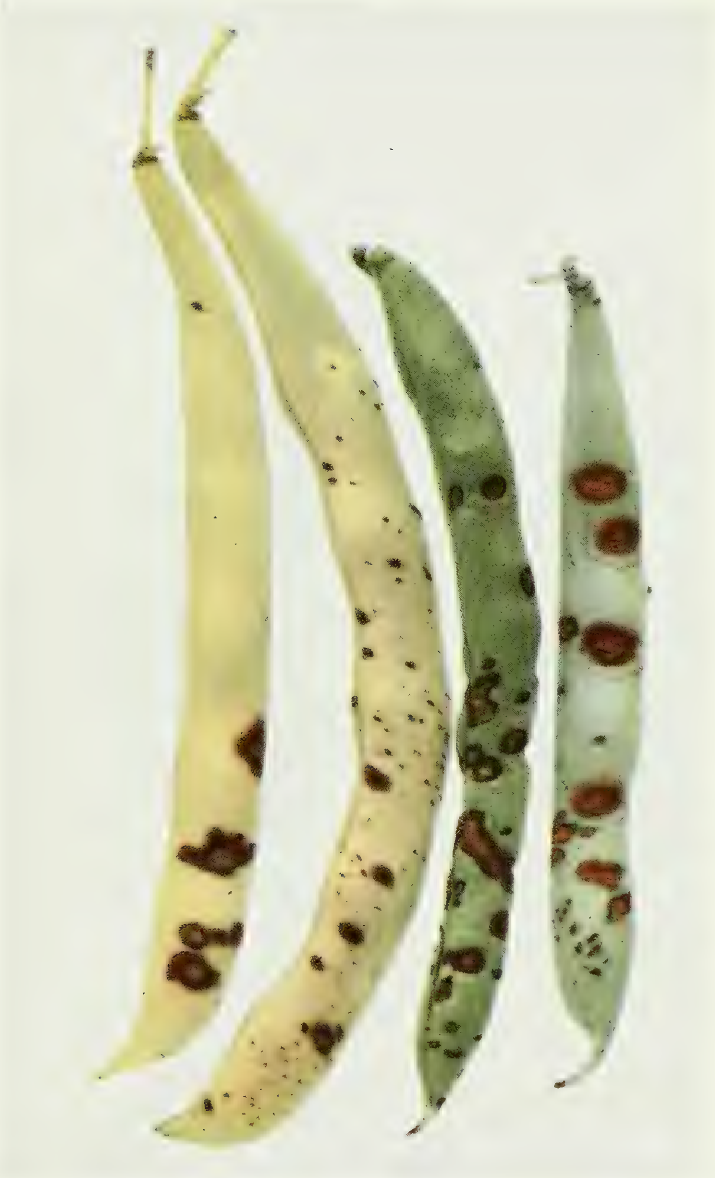
PLATE III





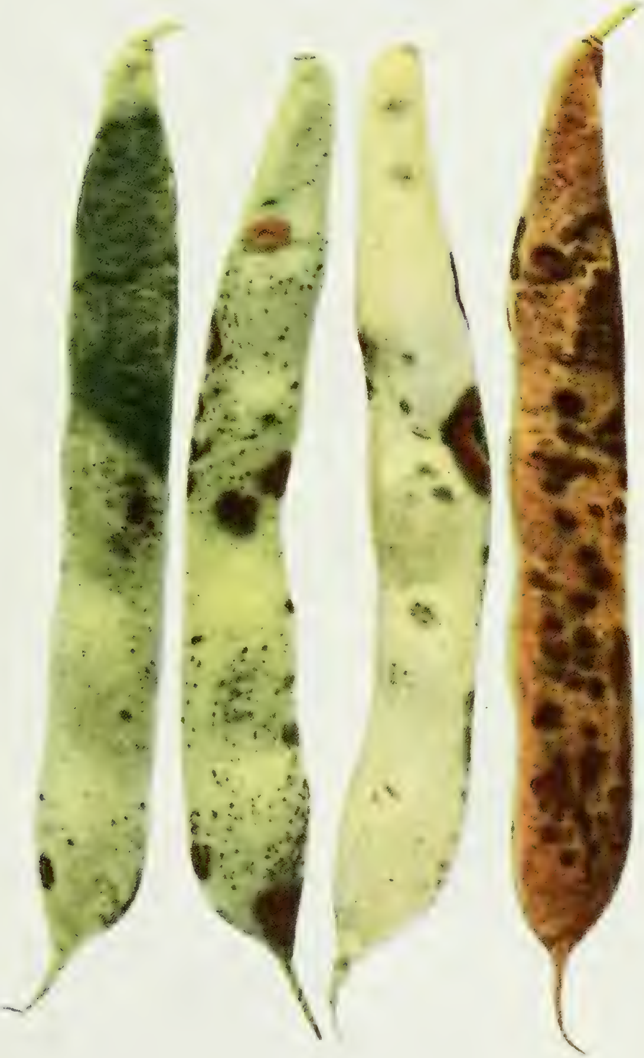
ANTHRACNOSE ON BEAN SEEDS

Upper left, Navy Pea; upper right, Red Kidney; lower left, White Marrow; lower right, Brockton

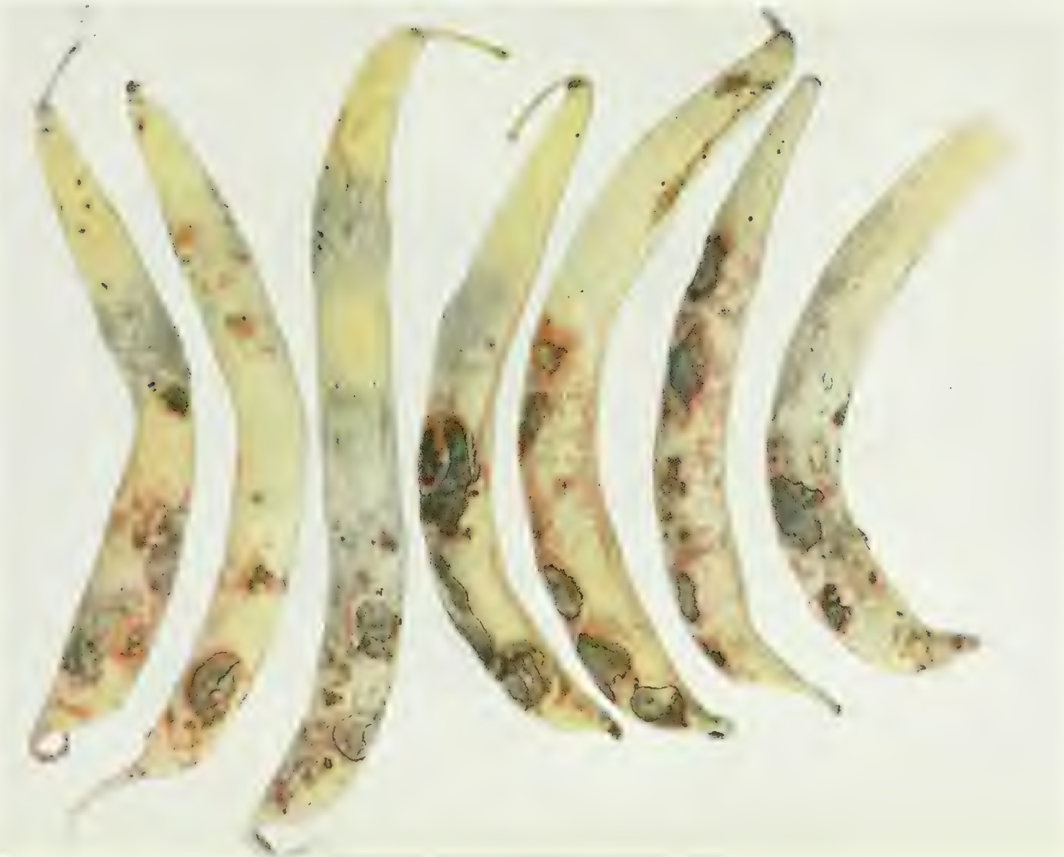


ANTHRACNOSE ON PODS OF WAX AND GREEN-POD BEANS

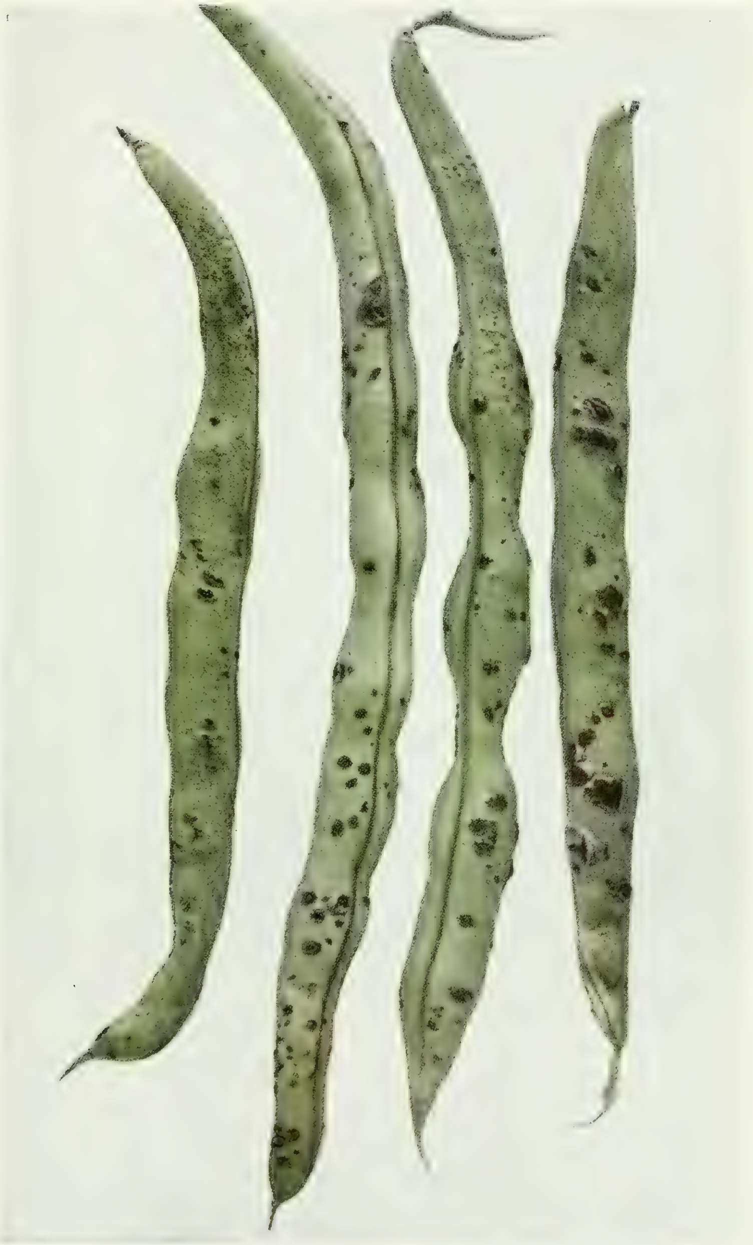
The acervuli can be seen in the larger lesions



BACTERIAL BLIGHT ON RED KIDNEY BEAN PODS



RHIZOCTONIOSE ON REFUGEE WAX BEAN PODS



RUST (UROMYCES APPENDICULATUS,) ON BEAN PODS

AUGUST, 1921 MEMOIR 45

CORNELL UNIVERSITY
AGRICULTURAL EXPERIMENT STATION

THE BOTRYTIS BLIGHT OF TULIPS

EDWIN F. HOPKINS

ITHACA, NEW YORK
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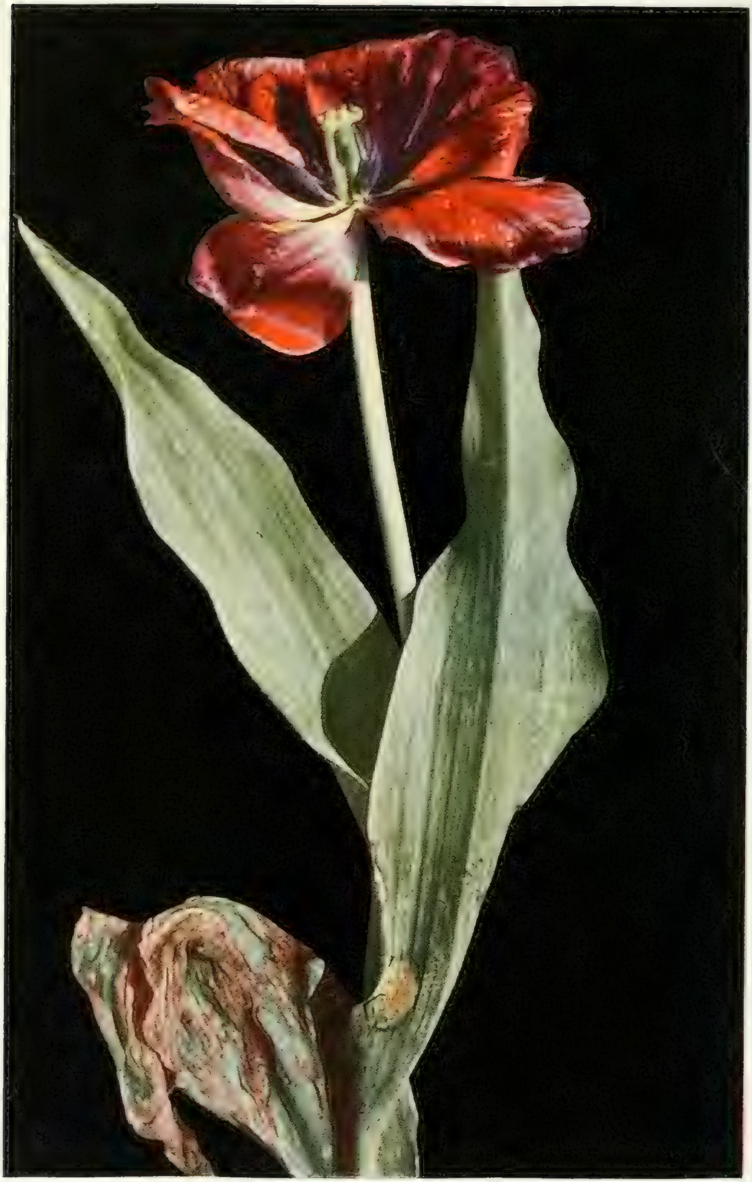
THE BOTRYTIS BLIGHT OF TULIPS



TULIP PLANT AFFECTED WITH BOTRYTIS BLIGHT

One-half natural size

THE BOTRYTIS BLIGHT OF TULIPS[1]

Edwin F. Hopkins

THE HOST PLANTS

It is reasonably certain that the Botrytis blight is restricted to plants in the genus Tulipa. This genus includes *Tulipa Gesneriana* L., the Darwin, or late, tulips, *T. suaveolens* Roth., the Duc van Thol tulip, embracing the early and forced varieties, and *T. sylvestris* L., the so-called wild tulip. The members of this genus have been under cultivation for so long that it is difficult to refer them to any natural species although the arrangement just given is commonly accepted (Bailey, 1917:3393-3394).[2]

Numerous references to the occurrence of this disease on other hosts have appeared in the literature. However, most of these statements are based on insufficient evidence, and show that the author had under consideration another Botrytis disease and did not attempt to verify his conclusions by cross inoculations. Ritzema Bos (1903a:20), for instance, says that while the disease affects other bulbs, such as hyacinths, gladioli, and certain iris species, the tulip is by far the most susceptible. Klebahn (1905:15-17) takes exception to this statement since he has found the disease on none of these plants except the tulip; furthermore, his experiments show the hyacinth to be immune. He seems to infer that Ritzema Bos was confusing two different diseases, and that possibly, on these hosts, the disease with which he was dealing was caused by *Sclerotium Tuliparum* Klebahn. However, Klebahn states that practical gardeners have told him that on ground which had borne bulbous begonias and dicentra no tulips came up.

Other notes frequently appear by authors who evidently confuse diseases caused by other species of Botrytis with the one under consideration, which is caused by *Botrytis Tulipae* (Lib.) comb. nov. Halsted (1891:352) gives a good example of this in attributing the disease on onions to this organism.

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[1] Also presented to the Faculty of the Graduate School of Cornell University, March, 1920, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

Acknowledgment. The author wishes to express his thanks to Professor H. H. Whetzel, of Cornell University, for his many valuable suggestions and for his helpful advice during the progress of the work.

[2] Dates in parenthesis refer to *Bibliography*, pages 359 to 361.

In order to gain some knowledge of the host range of the parasite concerned, numerous cross-inoculations were made by the writer. These experiments, which are summarized under the heading *Pathogenicity* (page 339), prove that the organism concerned does not attack certain of these hosts under artificial conditions, and therefore it probably would not under natural ones. Klebahn (1904, 1905, 1907) had previously made certain cross-inoculations tending to disprove the existence of a generalized type of parasitism in this pathogene.

The disease is restricted, under normal conditions, to the genus Tulipa; and while similar Botrytis diseases occur on the other hosts mentioned, and even on the tulip itself, they are not identical with this one.

VARIETAL SUSCEPTIBILITY

Varieties of both the early tulips, *Tulipa suaveolens*, and the late tulips, *T. Gesneriana*, are susceptible. Likewise, as already mentioned, the wild tulip, *T. sylvestris*, has been proved susceptible. The writer has collected diseased specimens of many varieties of the first two species and has seen the disease on the wild species.

Klebahn (1905:11), in his experiments, tested five varieties of tulips, presumably early varieties, but finding all susceptible he drew no general conclusion from this result. He thinks it would be desirable to compare the susceptibility of the early and the late species.

The writer found the disease on a large number of varieties of both species, and isolated the causal organism; he also succeeded in artificially infecting both species. He had almost concluded that there was not much difference in susceptibility. But in the spring of 1917, in a garden on the Cornell University campus where there was then a severe outbreak of the disease, one variety of late tulips (Baronne de la Tonnaye), which had certainly been exposed to the infection, showed no evidence of the disease. Up to the present time there has not been an opportunity to test this variety further.

THE DISEASE

NAMES

Various names have been applied to the disease. Ritzema Bos (1903a: 19) incorrectly used the name *kwaden plekken*, a term applied by Dutch bulb growers to soil that will not produce tulips. Later Klebahn (1907:3) showed that there are various causes of these "bad spots," but that usually they are due to *Sclerotium Tuliparum*. Hence the term *kwaden plekken* may not be applied specifically to this disease.

Ritzema Bos (1903 a: 19) uses also the word *Umfallen* to designate the disease as it occurs on tulip tops because it often causes the stem to break over. The writer believes, however, that this name is better applied to a physiological disease of tulips described by Sorauer (1903: 265). "Tulip mould" is the term used by Massee (1899: 158) and also by Halsted (1902: 438). Later Jacob (1912) states that it is commonly known as "fire."

The writer would suggest the name *Botrytis blight* for this disease, since it causes a typical blight, and although there is another Botrytis disease of tulips, this is the more important one by far.

HISTORY AND DISTRIBUTION

The Botrytis blight of tulips was probably first described in 1830 by Madame Libert, in connection with an herbarium specimen (Crypt. Ard. No. 36). She evidently observed only the sclerotia. She describes the fungus from the sclerotia as *Sclerotium Tulipae* Libert. From her description it is evident that she had studied the sclerotia of *Botrytis parasitica* Cavara (Saccardo, 1888-89).

The disease was first carefully observed by Cavara (1888), in upper Italy. However, Ritzema Bos (1903 a: 26) thinks Wakker (1885: 22) had the same disease under consideration before Cavara's publication appeared. He called it "tulpenziekte." Ritzema Bos (1903 a: 25) states that the disease had been known in Holland for more than twenty years, but that he is not certain when it first became seriously destructive there. In 1890 affected tulip bulbs were sent to him from Norway. The work of Ritzema Bos, while in some respects not very accurate, was valuable in that it attracted attention to the importance of the disease. He began his studies in 1896 and published several papers on the subject.

Carruthers (1901: 246) notes the occurrence of the disease in Northamptonshire and Cambridge, England, in 1901. It was reported near St. Petersburg in 1911 (Elenkin, 1911). Klebahn (1904: 18) mentions its appearance in Hamburg, Germany, in 1902, and in other parts of Germany later. He has done some of the most important work in clearing up the confusion in regard to the various sclerotial diseases of tulips and their life histories.

The appearance of the disease in America was first recorded by Halsted (1902). He had received in 1901, from a grower at Cape May, New Jersey, a diseased specimen, the bulb of which had originally been obtained from Holland. He stated that the disease had also occurred the previous season (1902:438). Obviously, therefore, it was introduced directly from Holland.

In a letter to Professor H. H. Whetzel, of Cornell University, Professor W. J. Morse, of the Maine Agricultural Experiment Station, mentions some unpublished records of the appearance of the disease at Barrington, Nova Scotia, in 1904, at Cobourg, Ontario, in 1906, and at Cartine, Maine, in 1910. There are also later notes. Professor Whetzel and the writer have received or collected specimens from Amsterdam, Sassenheim, and Aalsmeer, in Holland; from Germany; from Ithaca, McGraw, Garden City, Jamaica, and Brooklyn, in New York State; from Madison, Wisconsin; from Manistee, Michigan; from Washington, D. C.; from Bellingham, Washington; and from Carthage, Missouri. There is no doubt that the disease is widespread throughout the United States and Canada, and it probably will be found wherever tulips are grown.

ECONOMIC IMPORTANCE

There are apparently no exact figures available regarding the amount of damage from the Botrytis blight. Both Ritzema Bos and Klebahn speak of the great loss which this disease occasions to both field growers and florists. In fact, Ritzema Bos (1903: 91) was engaged for a time by a growers' association to investigate the disease.

Elenkin (1911) reports that in 1911, near St. Petersburg, Russia, 50 per cent of the tulip crop was destroyed by this and other tulip diseases. A collection and observations made by Whetzel at the New York Botanical Garden in 1916 show the Botrytis blight to have been severe there at that time. Occurrence of the disease is recorded also by Stout (1918:241), and letters and specimens from Dr. David Griffiths indicate that it was very prevalent in the Federal Government's bulb gardens at Bellingham, Washington, in 1917.

In the spring of 1917 the writer observed the disease in an epiphytotic condition. Most of the tulip tops in the ornamental beds in the Cornell University campus were severely attacked. These were late, or Darwin, tulips, and counts made on one variety, Spathulata, showed 100 per cent of the leaves diseased, of which 33 per cent were strongly infected and 67 per cent only slightly. Of the stalks, 98 per cent were diseased, 23 per cent severely and 75 per cent slightly. The bulbs all showed slight infections, but it is uncertain whether these were infections by *Botrytis Tulipae* or by *Penicillium* sp. However, in the variety Spathulata 4.6 per cent of unmistakable Botrytis lesions were found, and in the variety Mrs. Grover Cleveland, 5.2 per cent. The presence of sclerotia in these lesions made identification certain. These were probably lesions from the previous year and they show how small an amount of original inoculum is necessary to produce a severe infection on the tulip tops. Accordingly it is impossible to lay too much emphasis on the selection of clean bulbs. The writer believes that this is the most important disease of the tulip in this country.

SYMPTOMS

On the bulbs

On the brown outer skin, or husk, of the affected bulbs, small black sclerotia may frequently be found, about 1 millimeter in diameter. They appear also on the old, dried, flower stalk of the previous season, which sometimes remains attached to the bulb (fig. 22). The removal of this papery, brown skin often reveals lesions on the outer, white, bulb scale which might otherwise have escaped notice. These lesions vary from deep yellow to brown, are usually circular in outline, and have a definite margin which may be somewhat raised. The central part is ordinarily depressed and may have on its surface small black sclerotia (fig. 23). The lesions are formed sometimes at the apex of the bulb, sometimes at the base, but more often in the region between. Less frequently the sclerotia may appear white, which is due to their immaturity. By removing the outer, fleshy scale and examining its inner side, it will be seen that some of the lesions have penetrated almost to the inner surface. They rarely extend into the scales beneath.

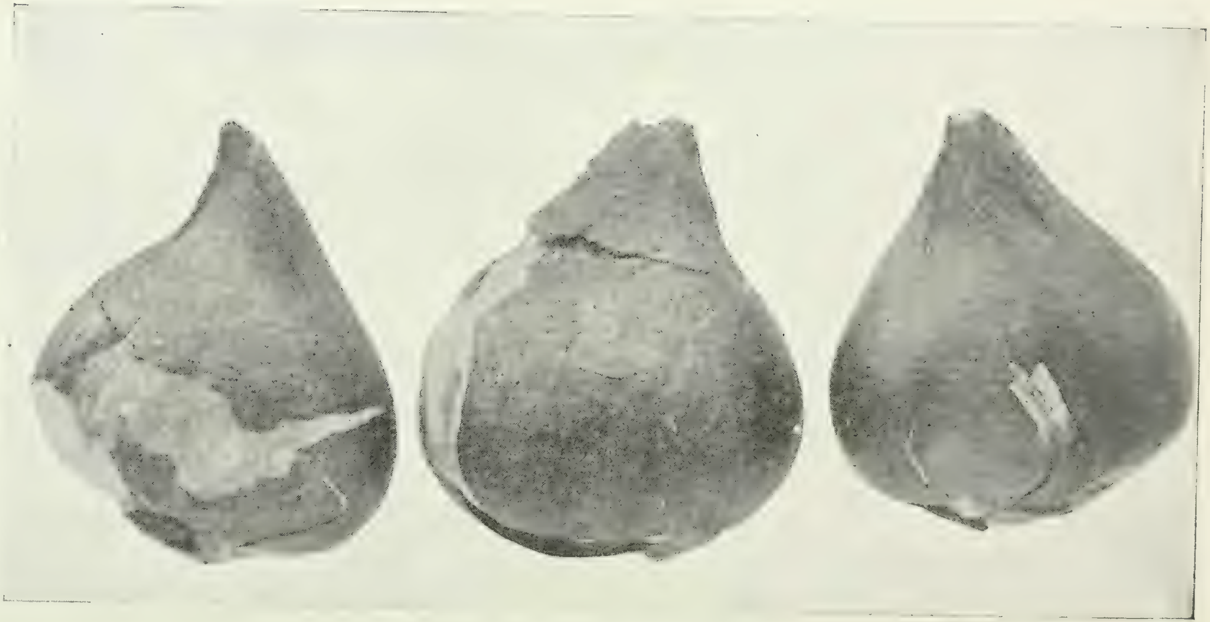

Fig. 22. SCLEROTIA OF BOTRYTIS TULIPAE

Showing them attached to the brown outer skin and the old, dried, flower stalk of dormant bulbs. Natural size

Under warm, humid conditions, a large part of the outer scale may become affected, and in some cases it is so densely covered with sclerotia that these coalesce and form a crust.

Care must be exercised not to confuse the lesions on the bulbs due to the very common Penicillium rot with those caused by *Botrytis Tulipae.* This is especially true of the incipient lesions which, in these two diseases, are often found almost impossible to differentiate. In general, the lesions of the Penicillium rot are more indefinite in outline and of a lighter yellow color, are usually raised and uneven, and do not, of course, have sclerotia on their surface. The appearance of green mold, which forms under favorable conditions, is a distinguishing characteristic.

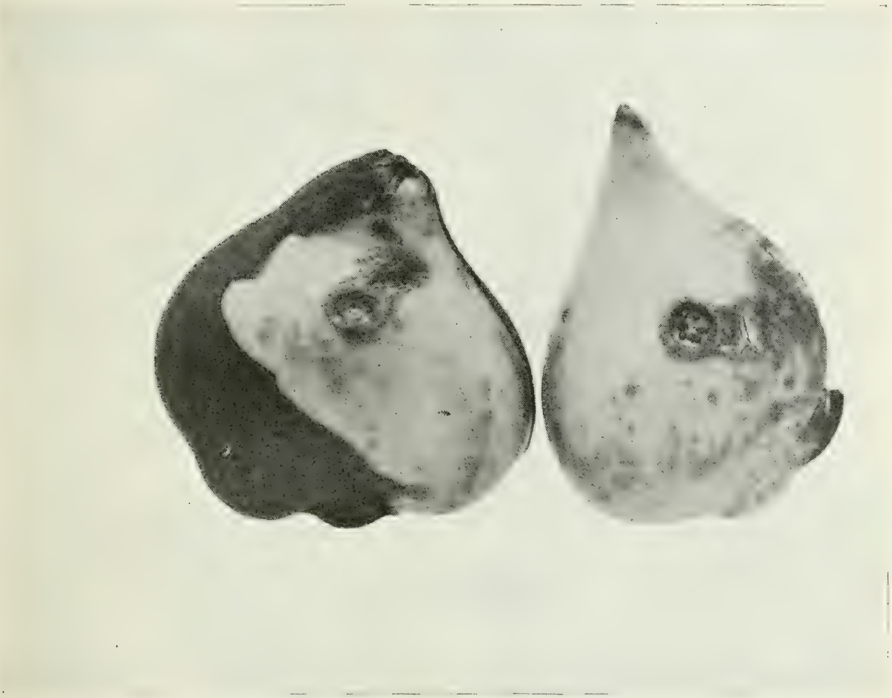

FIG. 23. BOTRYTIS BLIGHT LESIONS ON THE BULBS

The lesions in the outer, fleshy, bulb scale are depressed and show sclerotia on their surface. Natural size

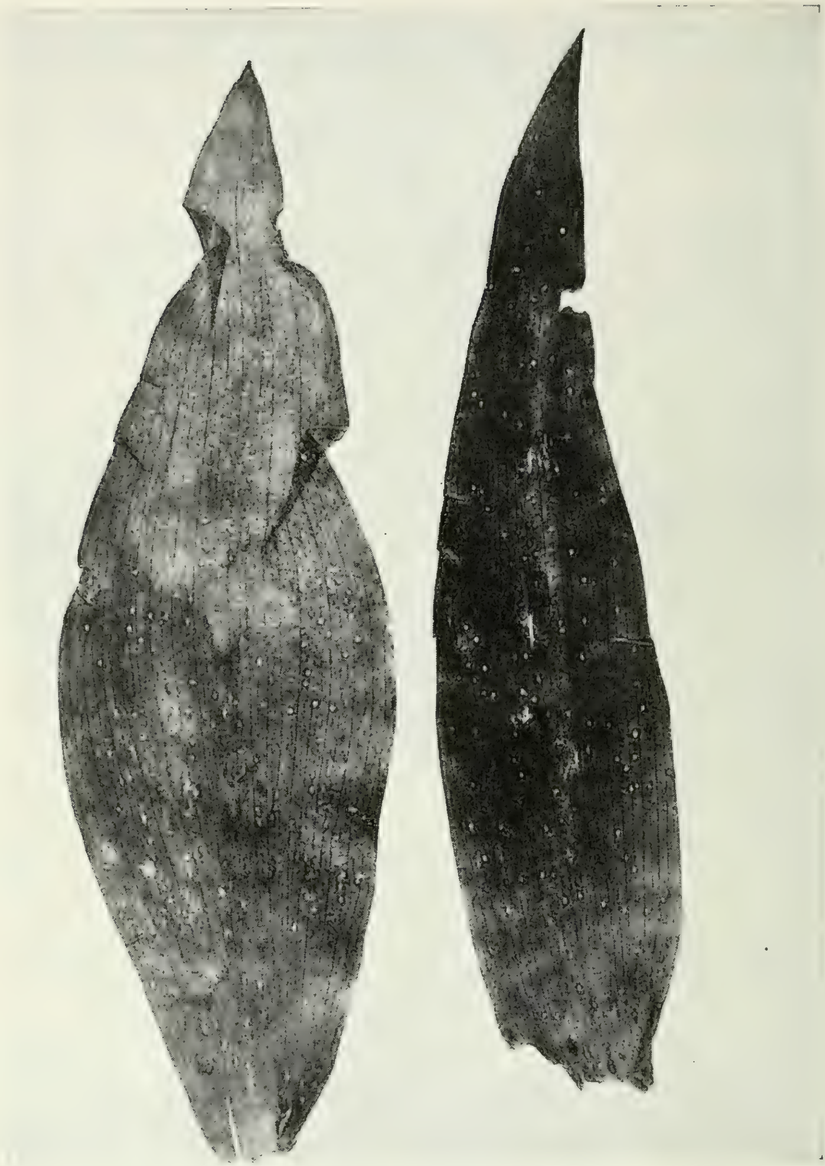

FIG. 24. BOTRYTIS BLIGHT LESIONS ON THE LEAVES

Young lesions. Three-fifths natural size

On the leaves

On the leaves the lesions show first as minute, yellowish spots, somewhat elongate in the direction of the leaf veins and surrounded by a darker, water-soaked area. They are slightly sunken and give the leaf a speckled appearance. As they enlarge, the areas become more depressed, the

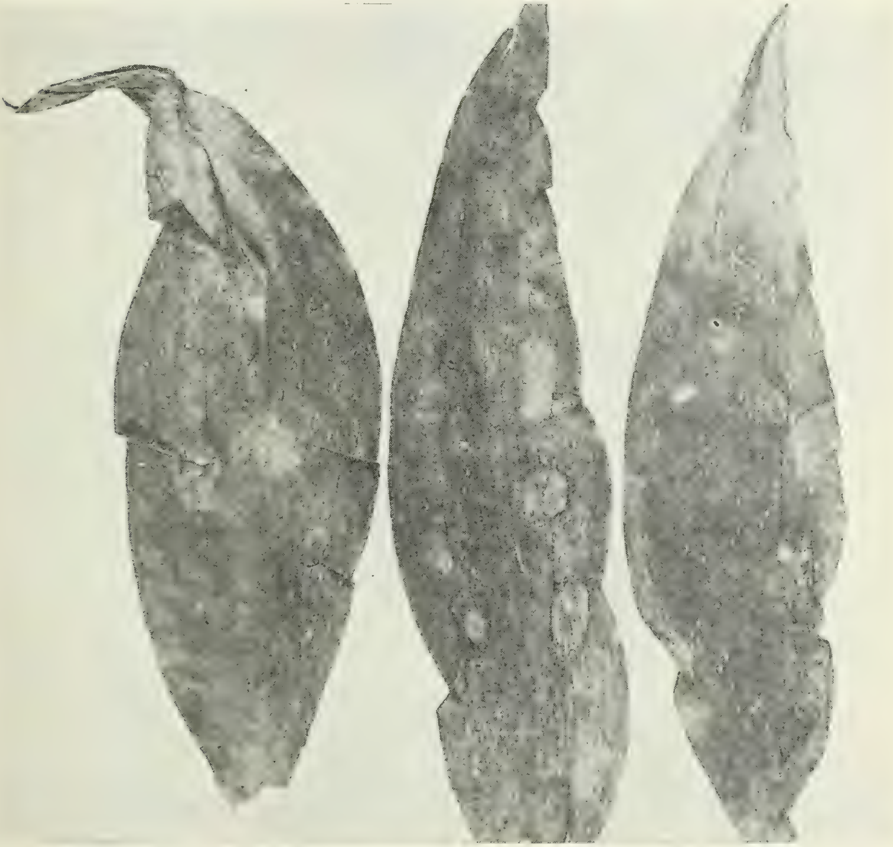

FIG. 25. BOTRYTIS BLIGHT LESIONS ON THE LEAVES
Lesions of a more advanced stage of development. One-half natural size

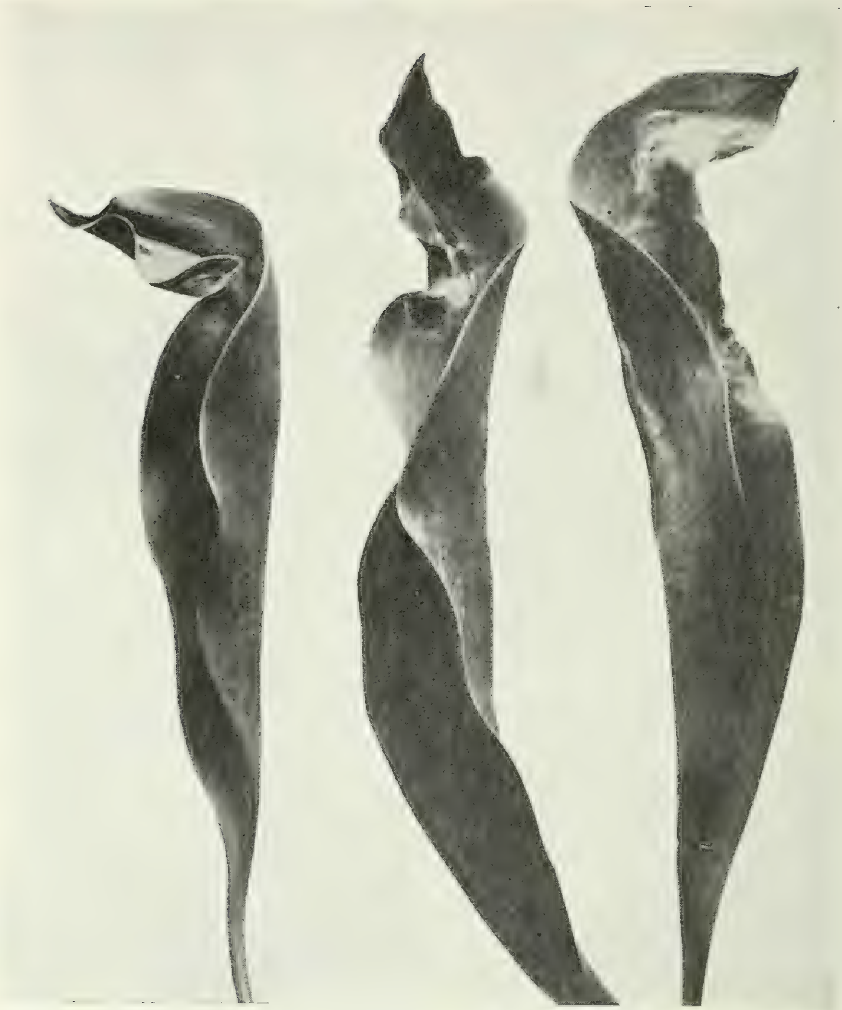

FIG. 26. BOTRYTIS BLIGHT LESIONS ON THE LEAVES
Peculiar twisting of leaves due to a marginal lesion. Natural size

color changes to a whitish gray with a brownish tinge, and a translucent or water-soaked area appears about the margin. At this stage the margins of the lesions are quite definite (fig. 24). Toward the center abundant conidiophores are often produced. Under favorable conditions the lesions enlarge still farther, coalesce, and frequently involve the entire leaf. If a lesion develops toward the base of a leaf, it may cause the leaf to break over. When an infection takes place on the margin of the leaf near the tip, there results the characteristic appearance shown in figure 26. This wrinkling and bending of the leaf to one side is due to the more rapid growth of the healthy tissue opposite the lesion. (Klebahn, 1905:4).

Both young and old lesions are found on the same leaf showing that infections take place continuously (fig. 25). The outer sheathing leaf is likely to be attacked before the others, probably being infected as it emerges from the bulb. It bends downward and usually is abundantly covered with conidiophores (fig. 27).

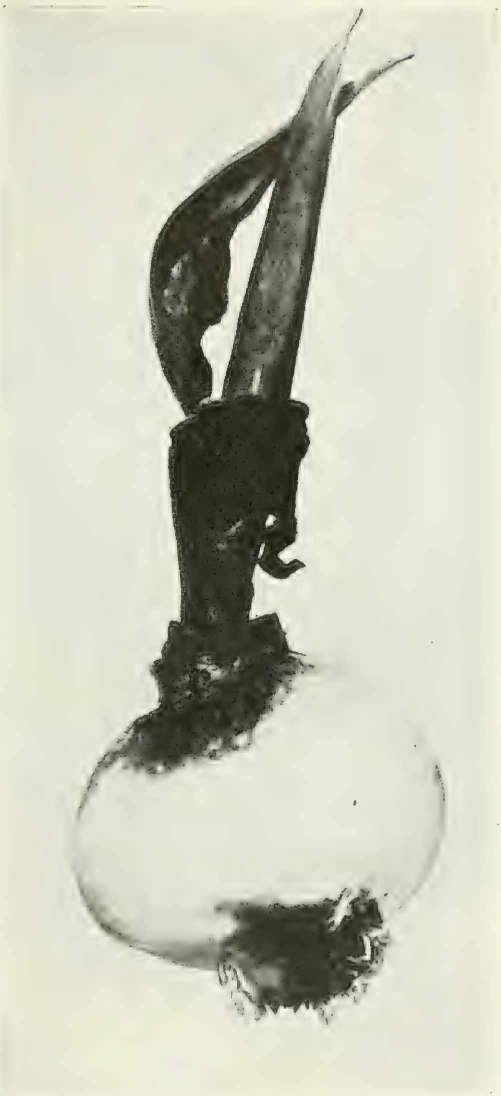

FIG. 27. A DISEASED PLANT

Showing the outer sheathing leaf infected by contact with a lesion at the tip of the bulb. Note the abundance of conidiophores which give rise to inoculum for secondary infections. Natural size

On the flowers

The lesions on the flowers (Plate XXXIII) are very striking, especially on red varieties of tulips. They begin as minute spots, whitish to light brown, the color being bleached from the perianth. These spots are evenly distributed over the surface and usually show no Botrytis fructification (fig. 28). After the lesions enlarge, however, they turn a deeper brown and involve the entire segment of the perianth, which finally becomes dry and wrinkled. At this time they show abundant conidiophores. The whole flower may be affected and appear blighted.

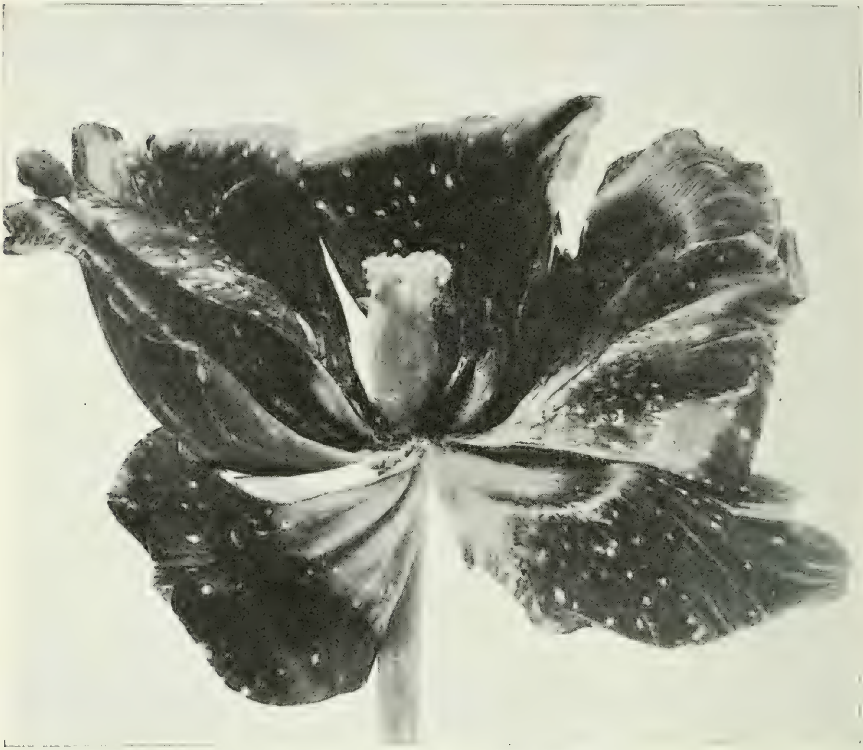

FIG. 28. LESIONS ON THE FLOWER
Natural size

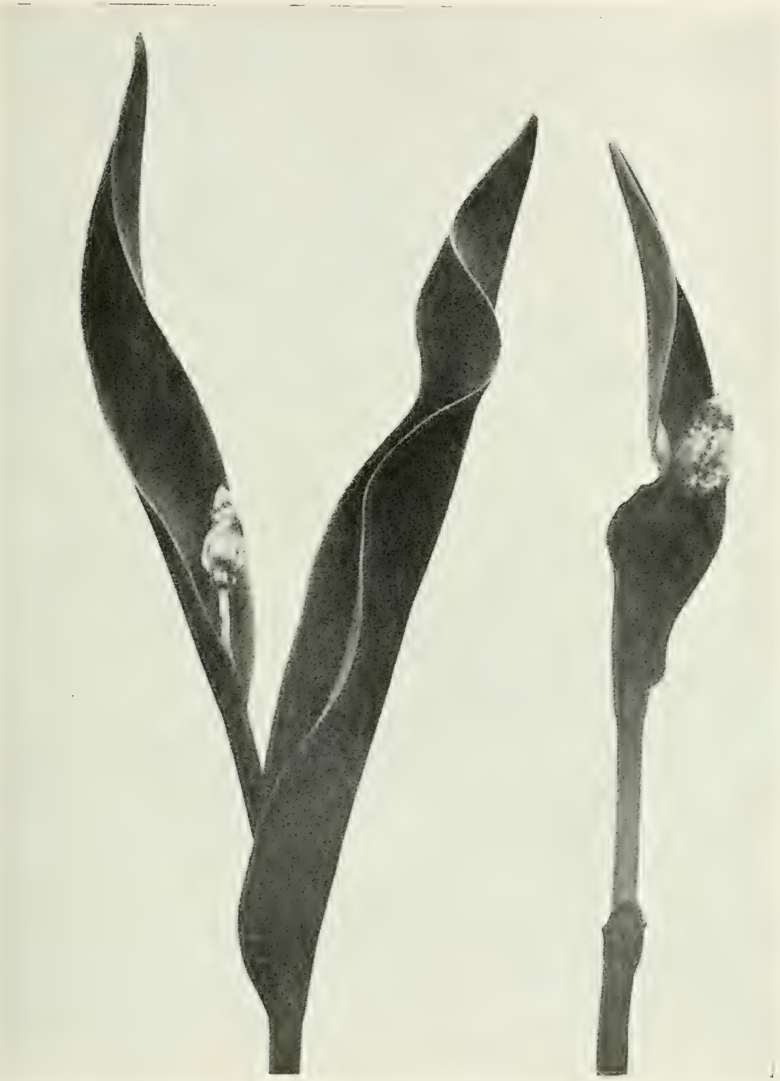

FIG. 29. BUD BLIGHT SHOWING THE BLANCHING OF THE INFECTED TISSUE
Natural size

Indeed, this blighting may take place when the flower is still in the bud and prevent it from opening. Such a typical bud blight is reproduced in figure 29.

On the stalks

While the lesions on the stalks are still small, they have much the same appearance as those on the leaves except that they are more elongate and more depressed. They are of a light brownish color in the center, and are surrounded by a water-soaked area. Older lesions near the base of the stalk appear as dark brown patches and often bear sclerotia on their surfaces, while those higher on the stalk, usually originating in the leaf axils, are grayish white and are covered with conidiophores. Both this blanching effect and the conidial layer are well illustrated in figure 30.

The extension of the lesion through the stem causes the latter to weaken at the point of attack and break over. If the lesion is near the base, the whole plant topples over; if it is higher up, the flower droops.

ETIOLOGY

Nomenclature

The tulip disease under discussion is caused by *Botrytis Tulipae* (Libert) comb. nov. A sclerotial form, referred to the form genus Sclerotium by Madame Libert, in 1830, belongs to this species. She called it *Sclerotium Tulipae* and was the first to describe it (Crypt. Ard., no. 36). As previously mentioned, she apparently did not observe the conidial form. Her original description, which the writer has not been able to see, is taken from Klebahn (1907:5) who quotes it as follows:

> *Sclerotium Tulipae*, N. Sparsum, adnatum, parvum, ovale, pallide fuscum, laeve, demum nigrum, rugosum, intus album. Ad caules, pericarpia et semina *Tulipae Gesnerianae*. Autumno.

In 1836 the species was again designated as *Sclerotium Tulipae* by Weinmann (1836:647). Sometime between 1841 and 1859 it was once more described in connection with an herbarium specimen, this time by Westendorp. He called it *Sclerotium entogenum* (Herb. crypt. Belg., no. 827). Finally Cavara (1888) described the fungus more completely,

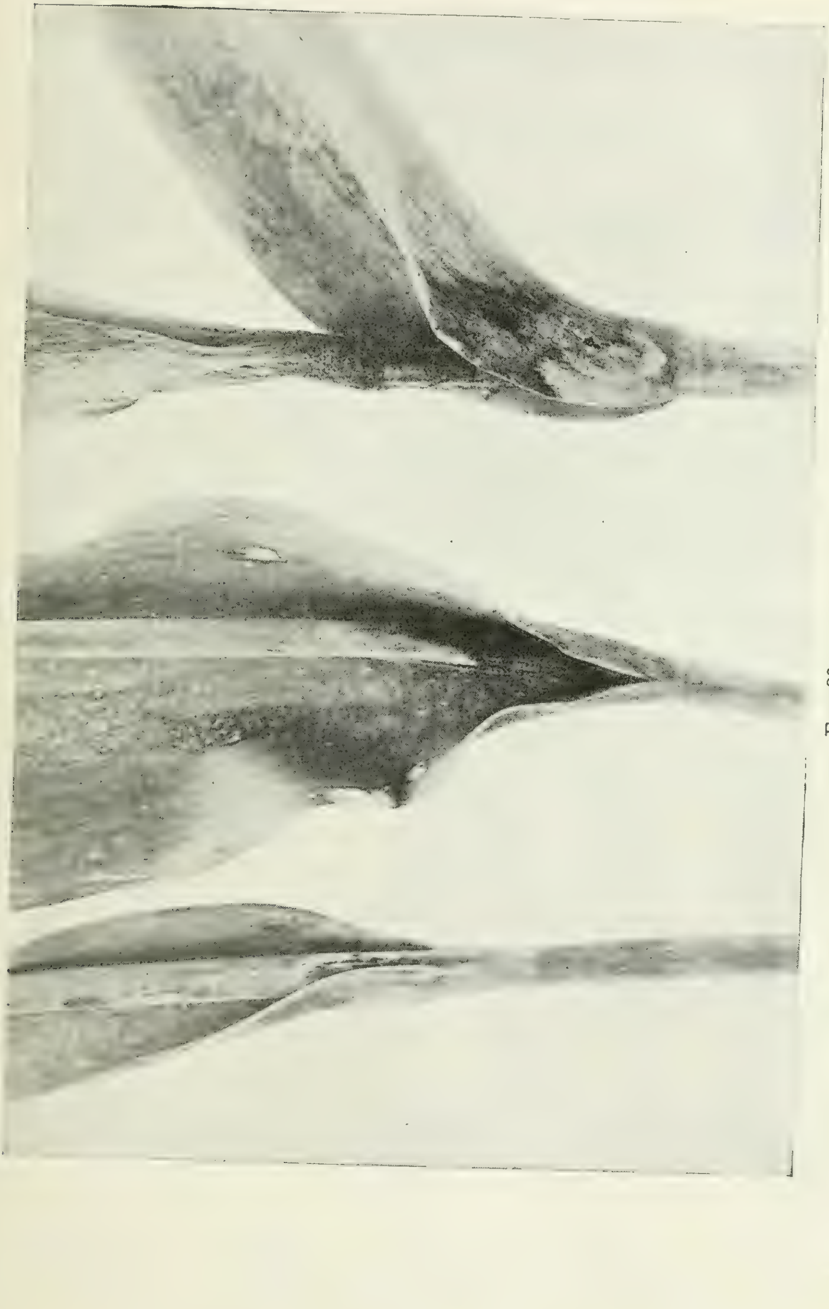

FIG. 30. DISEASED STALKS
Natural size

including both the conidial and the sclerotial stages, which he assumed belonged to the same species. His description is as follows:

> *Botrytis parasitica* nov. sp. Hyphis cinereis sparsis, erectis, articulo basali inflato; gonidiis ovatis, magnis, breviter pedicellatis, in ramulis minutis, capitatis, umbellatim dispositis; hyalinis vel dilute cinereis, 16–20 x 10–13 μ.
>
> Forma scleroziale.
>
> *Sclerotium Tulipae* Lib. Haemisphaericum, vel oblongum, nigrum, vix rugosum, superficiale vel immersum, intus albidum $\frac{1}{2}$–1 mm. latum.
>
> Hab. Ad folia, caules, petala, et capsulas *Tulipae Gesnerianae* in Horto botanico ticinensi.

Cavara (1888:432) justifies his description of this species as a new one on the basis of morphological differences and also because of its strong parasitic action. In his discussion of nomenclature, he says that *Sclerotium Tulipae* Terry, which infects tulips in the south of France, according to Saccardo (1888-89,) is probably a synonym of *Sclerotium cepivorum* var. *Tulipae* Desm., and perhaps is the same as *Scl. cepae* Desm. Cavara was not able to compare specimens of *Scl. Tulipae* Terry with *Scl. Tulipae* Lib. The writer, however, had an opportunity to examine a specimen of the former at the herbarium of the New York Botanical Garden. This specimen consists of three or four sclerotia with no adhering plant material. The sclerotia are large, however, and are not those of *Botrytis Tulipae* (Lib.) comb. nov. At the same place the author was able to see some of the collection of Cavara designated *Botrytis parasitica* Cav., and found it to be identical with his own collections.

Massee (1899:383) describes the organism as *Sclerotinia parasitica*. This description was not based on a perfect stage and in Massee's key is placed under "Conodial form only known." Since there is no evidence that the species under consideration is a Sclerotinia, this name is not valid. Massee's description is as follows:

> *Sclerotinia parasitica*, Massee; *Botrytis parasitica*, Cavara. Conidiophores grey, scattered, erect, basal joint inflated; conidia obovate, large, shortly pedicellate, on short umbellately arranged branchlets, hyaline or tinged grey, 16–21 x 10–13 μ; sclerotia formed in the parenchyma of the host, globose-depressed, smooth, greyish, then black, 2–3 mm. diam., sometimes numerous, and forming black crusts.
>
> Botrytis on leaves, stem, and flowers of cultivated tulips; sclerotia more especially on the bulbs.
>
> *Distr.* Holland, Britain.

A consideration of these facts has led to the following designation of the species:

Botrytis Tulipae (Libert) comb. nov.

Sclerotium Tulipae Lib. Crypt. Ard., no. 36. 1830.
Sclerotium Tulipae Weinn. Hym. Ross., p. 647. 1836.
Sclerotium entogenum West. Herb. crypt. Belg., no. 827. 1841–1859.
Botrytis parasitica Cav. Appunti die Pathologia Vegetale. Ist. Bot. R. Univ. Pavia. Atti 2:1:432. 1888.
Sclerotinia parasitica Massee. A text book of plant diseases, p. 383. 1899.

Botrytis Tulipae (Libert) comb. nov. may be briefly described as follows: Mycelium variable in diameter, often anastomosing, branches not constricted at the base; conidiophores arising directly from the mycelium, erect, brown in color, proliferating, twisting on their axils when dry, slightly swollen at the base; branches of conidiophore arising at an angle of about 60 degrees, dichotomous, not streptiform, apices swollen; conidia large, 12-24 x 10-20 μ, obovate, reddish brown in mass; microscopically gray to hyaline, smooth with a short stalk, often or commonly not remaining attached; sclerotia at first white, finally black, small, 1-2 millimeters in diameter, circular or somewhat elliptical in outline, flattened vertically and often convex. Microconidia globose, about 3 μ in diameter, occurring on special penicillate, obclavate conidiophores arising in white tufts from the substratum (fig. 40). Parasitic on *Tulipa* spp.

Cavara (1888:432) says that *Sclerotium entogenum* West. develops on the stalks of asparagus and does not differ greatly from *Scl. Tulipae* Lib.; he says, moreover, that Westendorp thinks *Sclerotium entogenum* West. should be regarded as the type of the species. *Sclerotium Tuliparum* Klebahn is a species which must not be confused with *Botrytis Tulipae.* It is a large, sclerotial form with which no conidial stage has yet been connected.

The species must, therefore, still be classified among the Hyphomycetes of the Fungi Imperfecti. It is placed there by Lindau (1900:435) in the subgroup Mucedinaceae-Hyalosporae-Botrytideae.

Klebahn (1904:21), from his studies on *B. Tulipae*, seems certain of the connection of the sclerotia occurring on the tulip bulbs with the conidial form on the leaves. However, as he did not use pure-culture methods, it seemed desirable to clear up this point. Pure cultures were made by the writer from the sclerotia occurring on the bulb and from conidia on the leaves of the same tulip plant. These cultures were identical, and when inoculated into sterilized tulip leaves both produced normal conidia and sclerotia. Both also caused infection of healthy tulip plants.

Morphology

The mycelium shows no peculiar characteristics. It varies in diameter, depending on the conditions under which it lives. The branches are not constricted where they join the parent hypha. Anastomosing is frequent (fig. 31). The conidiophores, when mature, are deep brown in color except near the base, where they are hyaline. They are indeterminate in length, for under favorable conditions, after forming one head of conidia terminally, the main axis may proliferate and form another head (fig. 50). This may be repeated until finally there are several clusters of conidia on a single conidiophore (fig. 32). It should be remembered that although some of these clusters appear to be lateral, they are really formed terminally. When

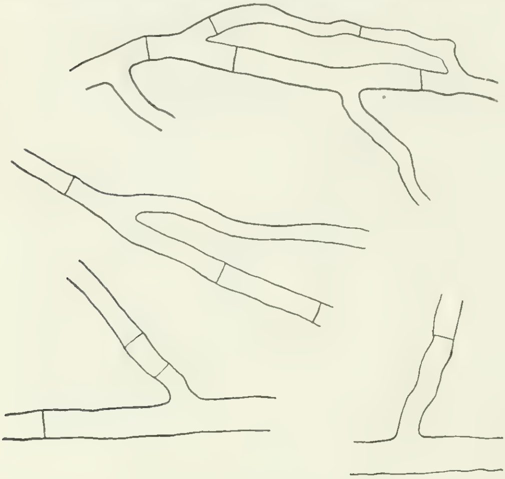

FIG. 31. MYCELIUM OF BOTRYTIS TULIPAE. × 600
Type of branching and anastomosing of the hyphae. (Camera-lucida drawing)

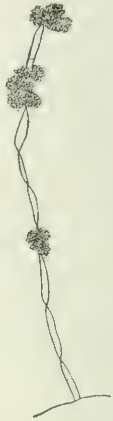

FIG. 32. CONIDIOPHORE OF BOTRYTIS TULIPAE. × 67

A conidiophore unmounted. The several clusters of conidia due to proliferation, and the twisting of the stalk when dry, are both visible. (Camera-lucida drawing)

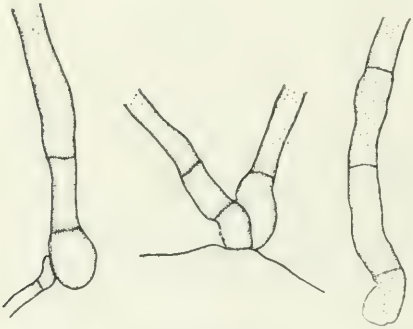

FIG. 33. SWOLLEN BASES OF CONIDIOPHORES OF BOTRYTIS TULIPAE. × 600
(Camera-lucida drawing)

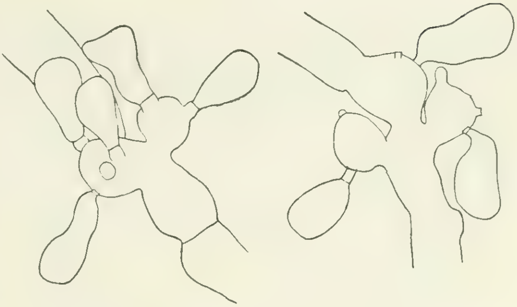

FIG. 34. ATTACHMENT OF CONIDIA OF BOTRYTIS TULIPAE. × 600

Camera-lucida outline of conidia shown in fig. 35. Proliferation of the conidiophore has taken place after the formation of conidia on the head.

dry, the conidiophore is flattened and twisted on its axis, and is slightly swollen at the base (fig. 33). The branches of the conidiophore rise from the main stalk at an angle of about 60 degrees, and their ultimate ends, which bear the conidia, are somewhat swollen. The conidia are produced on these swollen ends by a pushing out of the protoplasm in a bud-like manner, but they shortly assume a definite shape. When mature, they remain attached by short sterigmata (figs. 34 and 35). Various stages of conidial formation

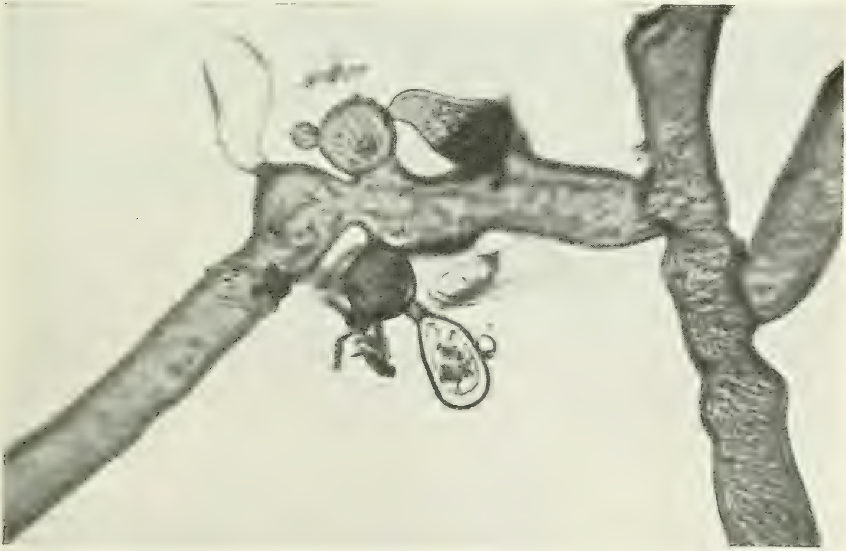

FIG. 35. ATTACHMENT OF CONIDIA OF BOTRYTIS TULIPAE. × 840

Note the short sterigma. These conidia are mature. (Photomicrograph) are shown in figures 36 and 37.[3] Usually young conidiophores were chosen for study because of the firmer attachment of the conidia. The preparation of the mounts required considerable patience because of the delicate attachment of the conidia, and oftentimes many mounts were prepared before one was obtained which showed clearly the details of structure. When young, the conidia are hyaline, but as they mature they assume, in mass, a brownish color. Microscopical examination shows that most of this color is in the spore wall. The conidia are obovate, and when shed, the short sterigmata may sometimes be seen still attached to the spores.

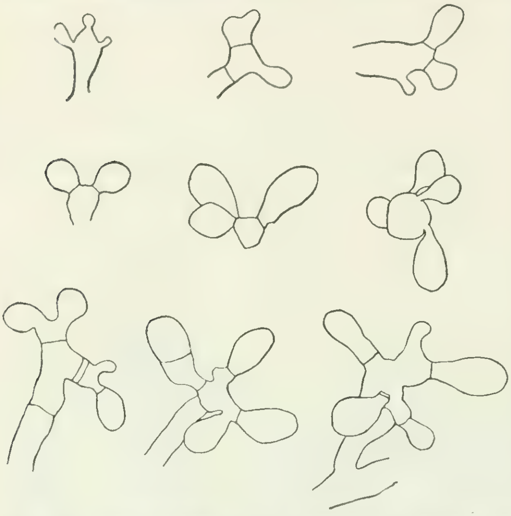

FIG. 36. DEVELOPMENT OF CONIDIA OF BOTRYTIS TULIPAE. × 600

Illustrating the bud-like manner of their formation. (Camera-lucida drawing)

The conidia vary considerably in length and to a less extent in width, but this variation does not depart greatly from a mean which is more or less constant. This is shown graphically in figure 38, which represents the measurements of one hundred spores. One curve expresses the variation of the spore length and the other that of the spore width.

The spores measured were from single-spore cultures and developed on sterilized tulip leaves in petri dishes. Abundant conidial fructifications were formed which were practically identical in appearance with those occurring in nature. They were mounted in the mounting fluid previously described[3] and were measured under the oil-immersion lens by means of camera-lucida drawings. One hundred spores were outlined and the outlines measured with a millimeter rule. The error in measuring was calculated to be less than 2 per cent. On the basis of these measurements the average limits of variation may be placed at 12-24 x 10-20 μ.

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[3] In studying the detail of the conidiophore, especially the attachment of the conidia, satisfactory mounts were obtained by first adding to the material on the slide a drop of 70-per-cent alcohol in order to "wet" the conidiophores rapidly. The material was then flooded immediately with a mounting fluid prepared by mixing equal parts of 2-per-cent potassium acetate in water and 40-per-cent glycerin in alcohol and then adding a trace of copper acetate. The excess mounting fluid was removed with filter paper and the mount covered with a cover glass. Such mounts keep very well, do not dry out, and may be kept permanently when ringed with balsam or gold size.

These figures do not show the distribution of the spores within these limits. From figure 38, however, it is apparent that the greater number have a length of 16-17 μ and a width of 9-10 μ. These measurements were checked with those of spores from another culture derived from a different locality. Measurements made of the spores of a large number of Botrytis specimens seem to show that one hundred conidia suffice to establish the mode for a given species.

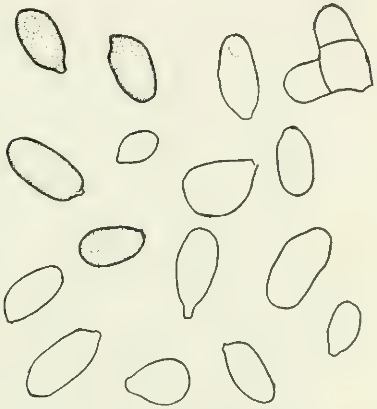

FIG. 37. DEVELOPMENT OF CONIDIA OF BOTRYTIS TULIPAE. × 600

Mature conidia. (Camera-lucida drawing)

The sclerotia as formed in a petri-dish culture, (fig. 39) are at first white, and later, a shiny black. They are circular, elliptical, or somewhat irregular in outline, flattened vertically, and often slightly convex. They might be described by the term "loaf-shaped." Ordinarily the sclerotia are about one millimeter in diameter. Their size may be considerably affected by the amount of drying to which they are subjected.

Physiology

Growth

Botrytis Tulipae grows very readily on the various kinds of media, both liquid and solid, on which it has been planted. In the writer's experimental work the commonest medium employed was potato-dextrose agar. On this the fungus makes a rapid, fluffy mycelial growth, which later becomes appressed to the surface of the agar and the sclerotia then begin to form. These are very numerous and are imbedded in a tough, mycelial membrane which covers the surface of the substratum. As mentioned under *Conidia production*, conidia are rarely formed in such cultures. Moreover, there is scarcely any color production in this medium.

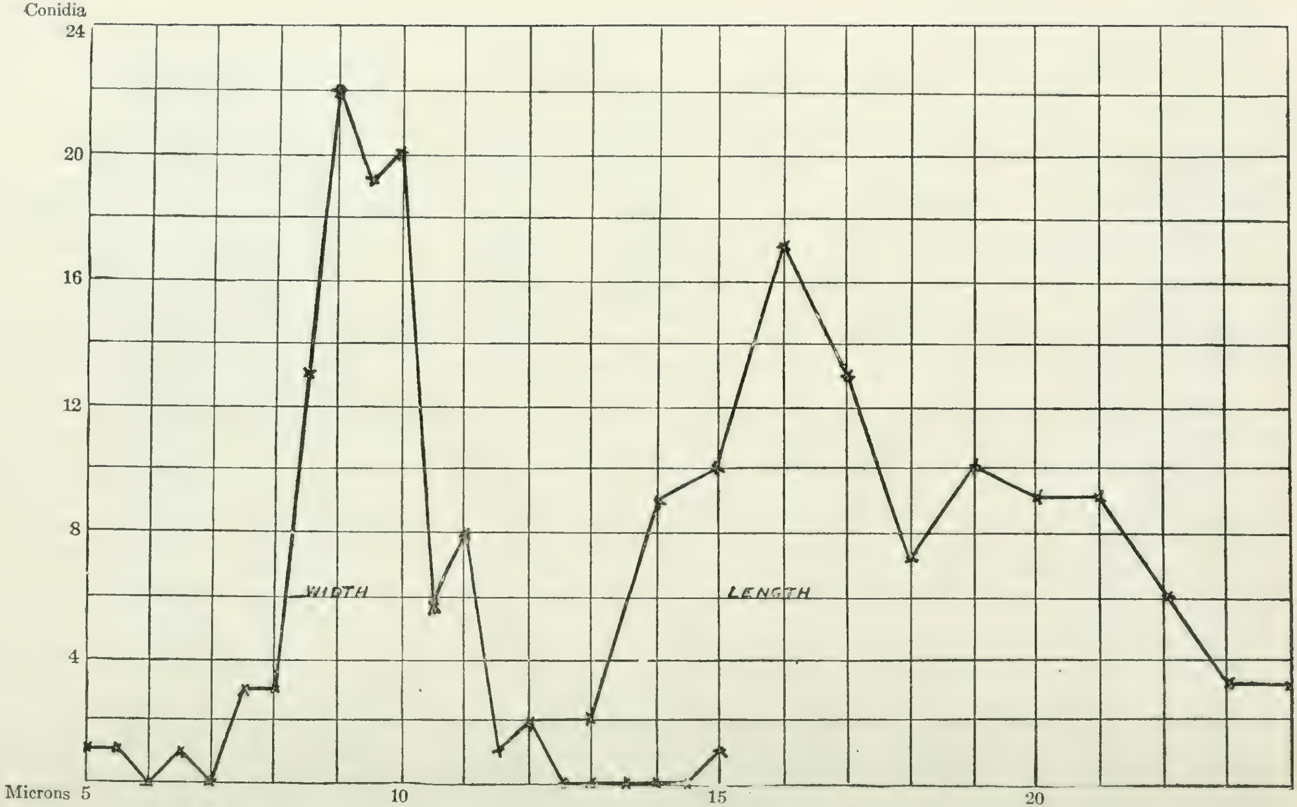

FIG. 38. GRAPHIC REPRESENTATION OF CONIDIAL MEASUREMENTS OF BOTRYTIS TULIPAE

Showing the distribution of 100 conidia. The ordinates show the number of conidia; the abcissae, their length or width in microns

It is of little importance to describe at length the growth on other media: sucrose is utilized as a source of carbon apparently as successfully as glucose; on a glucose solution, without mineral nutrition, the growth is very poor; on plain agar and water the growth is sparse and the mycelium tends to spread; but on agar containing a mineral-nutrient solution, with no source of carbon, an excellent growth takes place, even better, perhaps, than that on potato-dextrose agar. This indicates that the carbohydrates of the agar itself are being utilized. With oxalic acid as a carbon source, the growth is poor.

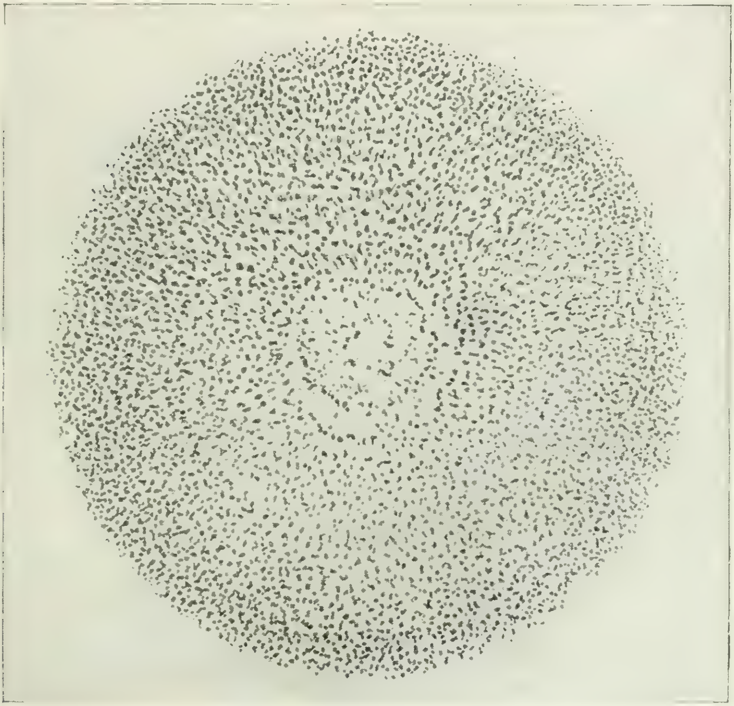

FIG. 39. SCLEROTIA OF BOTRYTIS TULIPAE

As formed in a petri-dish culture on potato agar. Natural size

Growth is best on an acid medium, and, although no experiments have been made to determine the range of acidity, good development has always been observed when the acidity was approximately +20 according to Fuller's scale. Perhaps the range is as wide as that determined by Munn (1917:407-408) for the growth of *Botrytis Allii.*

Conidia production

Although conidia are produced abundantly in nature on tulip leaves, in agar culture under the ordinary laboratory conditions they rarely appear. The fungus tends rather to form sclerotia. However, early in 1917 the writer observed for the first time that conidia had formed under these conditions when some conidiophores developed in five petri-dish cultures. It should be noted that these conidia were produced without any special treatment, and that, although they were produced once or twice afterward, this is rather unusual under the conditions obtaining in ordinary culture vessels.

On March 17, 1917, sterilized tulip leaves in large test tubes, 20 x 2.5 centimeters in size were inoculated with cultures of three different strains in duplicate. In two of these strains a fluffy mycelium soon developed, which spread along the leaves. After about ten days conidiophores began to develop, usually toward the top of the culture, and by March 31 there was an abundant development of conidiophores close to the surface of the leaves just beyond the aerial mycelium as well as on the mycelium itself. The conidia produced were able to cause infection in healthy tulips. This experiment was repeated on April 27, 1917, and the cultures were observed daily in order to note the first appearance of the conidia. This occurred in five cultures on May 8.

Some time later, Professor Whetzel suggested that a partial drying of the plate cultures, after a good growth of mycelium had started, might produce conidia. This experiment was accordingly performed, and the partial drying was accomplished by so placing the petri-dish cover as to expose part of the agar surface, thus permitting more rapid evaporation. The petri dishes were usually left overnight in a dust-free chamber and were tightly covered again the next morning. Conidia were in this way produced successfully in a large number of instances, not only from *B. Tulipae,* but also from other Botrytis species which do not readily fruit in culture. On halves of sterilized tulip bulbs in petri dishes the fungus was also found to fruit abundantly. There seems to be a relation between conidial production and the relative humidity over the culture, or, to state it more concisely, a relation between the rate of evaporation and conidial production.

There is, perhaps, something inherent in the nature of the host tissue which makes it an especially good substratum for the production of conidia; but it is more likely that the fungus finds in such cultures more variation in the moisture relations. This would explain why, in the experiments with sterilized tulip leaves, the conidia were not produced uniformly throughout the culture but only on a limited area toward the top of the culture tube, where the moisture relations were presumably most favorable.

Dissemination of conidia

The peculiar twisting of the conidiophores, already mentioned, is thought to have some relation to the dissemination of the conidia. In the first place, the conidia fall away very readily. This becomes obvious when an attempt is made to mount conidiophores bearing conidia in a liquid medium or if a conidiophore is jarred slightly. Secondly, the conidiophores are very hygroscopic, and a small change in the atmospheric moisture will cause them to twist with considerable violence and frequently even to dislodge the conidia. This phenomenon can be easily observed in this and in other Botrytis species by placing the specimen under a binocular microscope. Merely breathing on it gently suffices to produce these contortions. It may be concluded that in nature, owing to the frequent changes in humidity that occur, conidia are commonly dislodged in this manner and carried off by air currents.

The conidia are usually wind-borne. The writer has found that inoculations with conidia are best made by either blowing or dusting them on the host plants and subsequently spraying water on them with an atomizer.

Pathogenicity

The pathogenicity of *Botrytis Tulipae* was first demonstrated by Klebahn (1905:6), who inoculated tulip bulbs with sclerotia from a pure culture. However, he appears not to have done much exact inoculation work, and, with the exception of the experiment cited, did not use pure cultures in his infection tests. The writer does not consider that Ritzema Bos

(1903 a : 24) demonstrated the pathogenicity of the organism by his investigations, for he did not use pure-culture methods, but merely developed Botrytis conidia on leaves in moist chambers and then dusted the conidia on sliced bulbs, causing the bulbs thus treated to decay.

Experimental methods

The writer made isolations of the fungus from material obtained from many localities in Holland, Germany, England, Canada, and the United States. These isolations were made both from dry specimens (for it was found that the fungus would retain its vitality for a long time without moisture) and from recently infected plants. Moreover, they were made from sclerotia, mycelium, and conidia from various parts of the host — bulbs, stalks, leaves, buds, perianth, and stamens.

Although Klebahn (1905: 12) found it difficult to obtain pure cultures from the sclerotia, the writer has experienced little difficulty in isolating from sclerotia by the following method: The sclerotium is rubbed free from all adhering material with a clean piece of cheesecloth, and is dipped for instant in 95-per-cent alcohol to remove the surface film of air, that is, to wet the surface. It is then placed in a 1 : 1000 mercuric-chloride solution for about thirty seconds, after which it is quickly removed with sterilized forceps and placed in a drop of sterilized water in a sterilized petri dish. To thoroughly remove the mercuric chloride, the sclerotium is then rinsed in several successive drops of sterilized water in the same petri dish. Usually six washings are sufficient. The sclerotium, thus prepared, is cut into four pieces and planted on a poured plate of potato-dextrose agar. The whole operation should not take more than five minutes. The writer has used this method in isolating several hundred Botrytis specimens as well as specimens of other fungi, and rarely has a contamination occurred.

Fungi may be isolated from leaf tissue in this way if care is taken not to leave the material too long in the alcohol. In these experiments the mycelium was usually isolated from the leaf tissue and the stems by cleaning the epidermis with alcohol and then peeling it back, or often, when using stems and bulbs, by breaking or splitting them open so as to expose an uncontaminated surface. Small parts of the diseased tissue were then picked out with a sterilized, sharp-pointed scalpel and planted in agar.

Conidia were isolated in several ways. Sometimes they were blown over the surface of the agar from the bent end of a platinum needle. The needle was attached to a piece of glass tubing which served as a blowpipe. The tendency at first was to gather too many spores on the needle, but with practice a sparse sowing was readily made and transfers were then obtained from the resulting colonies. Another method was to pick off, with sharp-pointed forceps, a single conidiophore, under a binocular microscope if possible, and then touch it to an agar plate in several places. Pure cultures usually resulted from some of these plantings, and often all the cultures were pure.

Pure line cultures were obtained in two ways: first, by planting the fungus on a poured plate of plain agar and water, which caused the mycelium to spread out in its growth so that a single mycelial tip could be marked under the low power, cut off, and transferred; secondly, by the isolation of a single spore. In the latter method, which was the one most frequently employed, care was necessary lest more than a single spore should be obtained. A thin layer of agar containing a few conidia was poured into a petri dish and the spores were allowed to germinate slightly. After a conidium was marked and transferred to a poured plate, a microscopical examination was always made to ascertain positively that not more than one spore had been cut out. The growth of these cultures on potato agar is characteristic and is described under *Physiology* (page 355).

Both mycelium and conidia were used as inoculum. The mycelium inoculum was prepared by growing the fungus in a petri-dish culture until the colony had reached the size of an inch or so in diameter. Small cubes of agar containing mycelium were then cut with a sterile scalpel from the edge of the colony and placed on the plant part to be inoculated, with the side containing the mycelium against the host. To prevent the inoculum from drying out, the plants were either placed in a large, moist chamber or covered with a bell glass or a lamp chimney. When it was desired to injure the inoculated parts, this was done by pricking a sterile, sharp-pointed scalpel through the agar block into the host tissue.

In using conidia, difficulty was at first experienced in attempting to spray the plants with spore suspensions in water. No infections resulted. As already mentioned, this is explained by the fact that the conidia are not readily wet with water and consequently the water sprayed contained but few conidia in suspension. This was demonstrated by a microscopical examination of the drops on a slide sprayed with the suspension. Very few spores were found, and hence the chances for infection were slight. Since this method was unsatisfactory, an attempt was made to secure infection by dusting the conidia on with a camel's-hair brush. This proved to be very successful, and abundant infection resulted, both on dormant bulbs and on growing tulip plants. Plants inoculated in this manner were sprayed with sterile water from an atomizer and kept moist overnight.

Results of inoculations

The results obtained from inoculating dormant tulip bulbs with mycelium are shown in table 1. From this table it is clear that the dormant bulbs must be injured at the point of inoculation in order that mycelium may infect them. Briefly stated, the entire sixteen of the dormant bulbs which had been injured were infected by the inoculation, whereas none of the eight uninjured bulbs were thus affected. On the other hand, when ten bulbs were inoculated just after the flowers had been cut, all became infected, although only five were previously injured.

TABLE 1. RESULTS FROM INOCULATING DORMANT TULIP BULBS WITH MYCELIUM

Culture	Number of bulbs inoculated		Number of bulbs infected	
	Injured	Uninjured	Injured	Uninjured
B. 298	4	2	4	0
B. 163	2	1	2	0
B. 112	2	1	2	0
B. 149	2	1	2	0
B. XVII	2	1	2	0
B. XXVII	2	1	2	0
B. XXXII	2	1	2	0
Total	16	8	16	0

* The arabic numerals represent cultures from domestic sources, and the roman numerals, those of foreign origin.

A summary of the results obtained from inoculating tulip stems with mycelium appears in table 2. From this table it is evident that the tulip stems inoculated with twelve different cultures of *Botrytis Tulipae*, five

TABLE 2. RESULTS FROM INOCULATING TULIP STEMS WITH MYCELIUM

Culture*	Number of stems inoculated		Number of stems infected	
	Injured	Uninjured	Injured	Uninjured
B. 298	3	3	3	3
B. XXVII	4	5	4	3
B. XXV	4	5	2	2
B. XXVI	4	5	4	5
B. XVII	4	5	4	5
B. XXXII	4	5	4	5
B. 112	3	3	3	2
B. 143	3	3	3	3
B. 149	3	3	3	3
B. 150	3	3	3	3
B. 163	4	5	4	5
B. 414	1	2	1	1
Total	40	47	38	40

* The arabic numerals represent cultures from domestic sources, and the roman numerals, those of foreign origin.

TABLE 3. RESULTS FROM INOCULATING TULIP LEAVES WITH MYCELIUM

Culture*	Number of leaves inoculated		Number of leaves infected	
	Injured	Uninjured	Injured	Uninjured
B. XXVII	1	2	1	0
B. XXV	1	2	1	2
B. XXVI	1	2	1	2
B. XVII	1	2	1	2
B. XXXII	1	2	1	2
B. 163	1	2	1	2
B. 414	1	2	1	1
Total	7	14	7	11

* The arabic numerals represent cultures from domestic sources, and the roman numerals, those of foreign origin.

from foreign sources and seven from domestic, showed thirty-eight infections out of forty when pricked with a needle after inoculation, and forty out of forty-seven when not injured in this way.

A summary of the results obtained from inoculating tulip leaves with mycelium is given in table 3. All seven leaves showed infections when inoculated and injured; of the uninjured leaves, eleven out of fourteen showed infections.

Tulip flowers were inoculated with mycelium, with results as given in table 4. The tulip flowers, like the leaves, were all seven infected when an injury was made at the point of inoculation. Out of fourteen not so injured after inoculation, twelve showed infections.

TABLE 4. RESULTS FROM INOCULATING TULIP FLOWERS WITH MYCELIUM

Culture*	Number of flowers inoculated		Number of flowers infected	
	Injured	Uninjured	Injured	Uninjured
B. XXVII	1	2	1	1
B. XXV	1	2	1	2
B. XXVI	1	2	1	2
B. XVII	1	2	1	2
B. XXXII	1	2	1	2
B. 163	1	2	1	2
B. 414	1	2	1	1
Total	7	14	7	12

* The arabic numerals represent cultures from domestic sources, and the roman numerals, those of foreign origin.

It should not be deduced that the lower proportion of infections in the uninjured leaves, stems, and flowers was owing to the inability of the fungus to penetrate uninjured tissue. The writer attributes it rather to experimental error; for the inoculum was more likely to be lost or dried out before infection had opportunity to take place than when it was placed at once in such intimate contact with the host tissue as was the case when the latter was injured.

On April 3, 1917, six dormant bulbs were dusted with conidia from a pure culture of strain B. XXVII, and by April 25 five of these were strongly infected and some showed aerial mycelium arising from the lesions. On April 18, 1917, ten, clean, dormant bulbs were inoculated in the same manner with conidia from a culture of strain B. XXVII. In several days, nine of these showed numerous spots or streaks, varying in color from yellow to brown, where the conidia had been sown.

On March 30, 1917, at 5 p. m., four tulip leaves were dusted with conidia from a pure culture and the following morning at nine o'clock small water-soaked spots had appeared, a microscopical examination of the epidermis of which showed that the conidia had germinated and had penetrated the cuticle. On April 2 these spots showed a rusty color and were surrounded by translucent, water-soaked areas.

On April 1, 1917, at 4 p. m., three tulip plants in pots were inoculated with conidia of strain B. XXVII and placed under a large bell glass. On April 2, at 9 p. m., all had developed a considerable number of infections, as shown by the large number of small, yellow spots. These spots later become larger and of a somewhat reddish cast. About five days later the plants were severely diseased and showed a mycelial growth on the leaf surface. About seven days thereafter conidia were formed. On April 12 some sclerotia were noted in the leaf tissue.

On April 13, 1917, eight tulip plants were inoculated with conidia of strain B. XXVII and placed in a large moist chamber. On April 16 all showed numerous yellow-to-reddish spots on the leaves. A few similar spots appeared on the stems. By April 18, the small spots had coalesced and appeared as large, reddish lesions, some of which were covered with abundant conidiophores.

The inoculations with conidia are summarized in table 5. The plants were not mechanically injured at the time of inoculation.

TABLE 5. Results from Inoculating Uninjured Tulip Plants with Conidia

Experiment	Culture	Plant part	Number inoculated	Number infected
16	B. XXVII	Bulb (dormant)	6	5
18	B. XXVII	Bulb (dormant)	10	9
14	B. XXVII	Leaves (detached)	4	4
15	B. XXVII	Tops	3	3
17	B. XXVII	Tops	8	8

In using mycelium as inoculum on the leaves and the stems, the lesions, after a short time, become as typical as those of the same age produced in nature. In the beginning, however, although they show the characteristic yellowing, they are not exactly like natural lesions, for they assume the shape of the agar block used in the inoculation. As the infection spreads, the region about the inoculum becomes water-soaked, then depressed, and finally dried out. At the last stage, the lesion assumes a dull gray color and produces fluffy mycelium and sometimes also a conidial layer. The lesions tend to elongate in the direction of the stem and the leaf. If an inoculation is made on the edge of a young leaf near the tip, the peculiar twisting described on page 323, under the heading *Symptoms*, results. The whole plant top may be involved as a result of such an inoculation (fig. 42). On the dormant bulbs, also, the lesions are typical (fig. 41) with a dark brown, shiny surface. In using conidia as inoculum, the lesions are typical from the start and are essentially as described on pages 319 to 328.

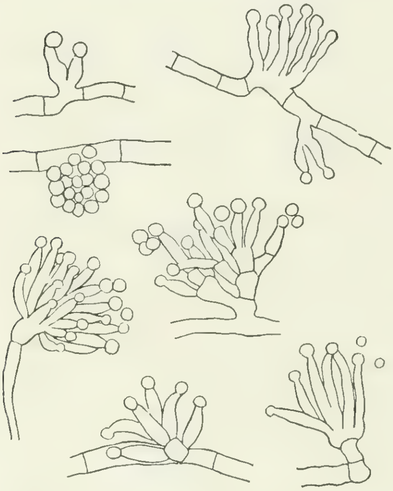

FIG. 40. MICROCONIDIA OF BOTRYTIS TULIPAE. × 600
(Camera-lucida drawing)

With but few exceptions, when infection was positive, the fungus in these experiments was re-isolated in pure culture by one of the isolation processes described under *Methods*, and checked identically with the original culture. Usually the tissue-planting method was the one employed.

In order to determine the range of parasitism of this species, a considerable number of experiments were made on both closely related and distantly related plants to find out whether *Botrytis Tulipae* is able to infect them.

The general results of these experiments appear in table 6. Certain of these, however, should be discussed more fully.

TABLE 6. RESULTS OF INOCULATION ON VARIOUS HOSTS FROM INOCULATING PLANTS NEARLY AND DISTANTLY RELATED TO THE TULIP

Plant	Inoculum	Number inoculated		Number infected	
		Injured	Uninjured	Injured	Uninjured
Lily of the valley........	Mycelium......	14	0	0	....
Onion (leaves)..........	Mycelium......	3	3	3	0
Onion (bulbs)...........	Mycelium......	16	1	0	0
Onion (stems)..........	Mycelium......	2	0	0	....
Lilium sp..............	Mycelium......	6	6	0	0
Narcissus (leaves).......	Mycelium......	11	11	11	0
Narcissus (leaves).......	Conidia........	0	5	....	0
Narcissus (stems).......	Mycelium......	3	3	3	0
Hyacinth (leaves).......	Conidia........	0	5	....	0
Crocus (tops)...........	Mycelium......	7	7	7	0
Crocus (bulbs)..........	Mycelium......	5	5	5	1
Gladiolus...............	Mycelium......	10	0	0	....
Peony..................	Mycelium......	19	0	3	....
Potato.................	Mycelium......	21	0	0	....
Golden seal.............	Mycelium......	6	0	0	....

From table 6, the relation of *B. Tulipae* to certain plants is evident: it is not able to attack at all the lily of the valley, the lily, the gladiolus, the potato, or the goldenseal, and, probably, not the peony. However, this relation will be made clearer by a discussion of certain observations made during the attempts to cause infection on these plants.

Inoculation of onion.—Leaves and stalks of onion plants were inoculated with three different cultures, one of these a typical culture of *B. Tulipae.* In those plants that were injured at the time of inoculation, strong infection took place on the leaves but none occurred on the stems. On the uninjured plants there was no infection. To serve as checks, other plants were inoculated at the same time with

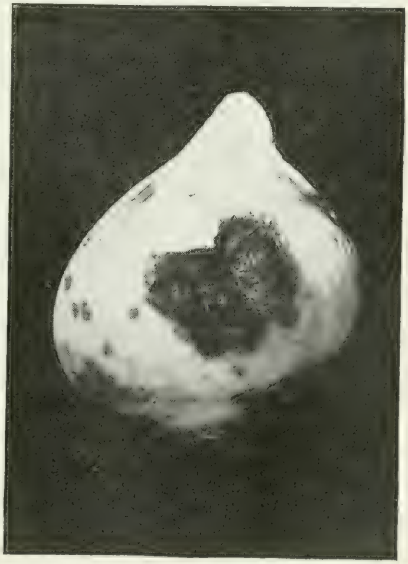

FIG. 41. RESULT OF ARTIFICIAL INOCULATION

Lesion on dormant bulb. The bulb was injured at the point of inoculation. Natural size mycelium from a culture of the onion Botrytis, *B. Allii* Munn, and with a large sclerotial form of Botrytis from tulips — not *B. Tulipae.* From both of these inoculations a strong infection resulted on the leaves and a slight infection on the stem, in the uninjured as well as the injured plants. A similar experiment with mycelium from the same cultures was tried on onion bulbs, and here *B. Tulipae* produced no infection, while the other two species produced a strong infection, but only on the injured bulbs. It is interesting to note here that on the uninjured bulbs inoculated with *B. Tulipae*, peculiar depressions appeared in the bulb scale that at first seemed to be slight infections. However, microscopical examination showed that the mycelial threads had not penetrated. They were merely superficial. Nevertheless, beneath the mycelium some epidermal cells and other deeper-lying cells had been killed. Microscopical sections of the injured bulbs showed mycelium in the punctures made by the scalpel. It had not, however, penetrated laterally into the tissue, although some of this tissue had been killed.

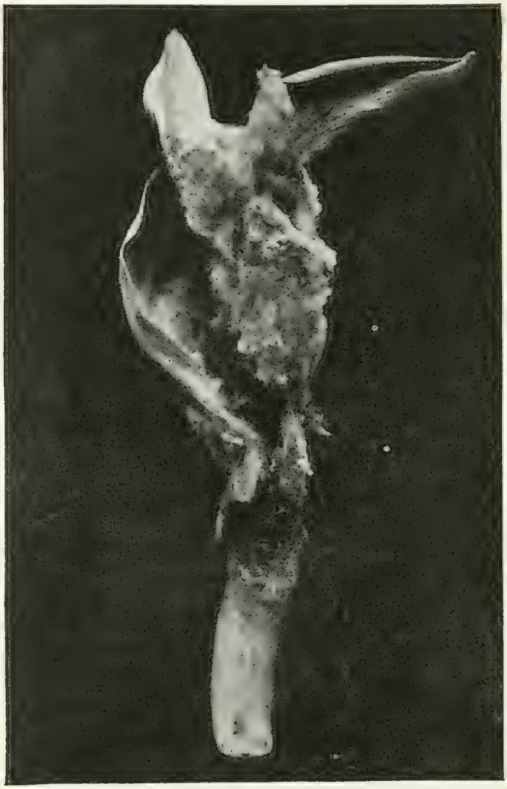

FIG. 42. RESULT OF ARTIFICIAL INOCULATION
The whole top is involved. Natural size

Inoculation of narcissus.— The result obtained on the narcissus was similar to that on the onion: strong infections appeared on the leaves and slight ones on the stems when the inoculation was performed on mechanically injured plants. Uninjured plants were not infected. When conidia were used to inoculate narcissus, there was no sign of resultant injury, and microscopical examination of the epidermis showed the conidia to be present but not germinated.

Inoculation of hyacinth.— Detached hyacinth leaves were inoculated with conidia from pure culture. On the fifth day after inoculation, small, yellowish, depressed spots appeared. Microscopical examination of these spots showed an abundance of germinated conidia but no penetration of the epidermis by their germ tubes. Moreover, the tissue beneath the epidermis showed no mycelium.

Inoculation of crocus.— Slight infections were caused on mechanically injured crocus leaves by mycelium of *B. Tulipae*, but no infection occurred on uninjured leaves. A large sclerotial Botrytis from tulip caused no infection in either case. Very slight infections were produced on the papery scales of crocus bulbs. On injured bulbs, all the five inoculated were infected; on uninjured bulbs, only one out of five was infected. Microscopical sections of these lesions showed mycelium ramifying through the tissue, and disintegration of the cells was observed.

Discussion of parasitism

It is evident from these pathogenicity experiments that *B. Tulipae* is practically restricted to tulips. Although under certain conditions it attacks some closely related plants, even such infection occurs, almost invariably, only when there is an injury made at the point of inoculation. Furthermore, a large number of injured plants failed to become infected. When we consider the ease with which the tulip may be infected, whether mycelium or conidia be used as inoculum and whether the host plant be injured or uninjured, these apparent exceptions only make more evident its restricted parasitism. Indeed the writer believes that in those instances in which *B. Tulipae* is reported on other hosts, if the fungus were really that species, the infection took place on an injured part of the host plant. On the other hand, the pathogene shows gradation in parasitism in its feeble attempts to invade plants other than its normal host. First, there are plants such as the crocus, on which are produced only slight infections which do not spread. Next, there are plants such as the narcissus and the onion, on which the conidia do not even germinate and infection by mycelium can begin only at an injured place. Again, there are plants such as the hyacinth, on which the conidia will germinate and cause local injury, without actually invading the plant. Finally, there is the tulip, in the case of which infections take place easily on uninjured plants.

While this series is too incomplete to be conclusive, the tendency shown is clear, and further experiments in this direction would probably furnish additional evidence of the very limited range of the parasitism of *B. Tulipae.*

Life history

Primary inoculation and infection

The fungus survives the dormant period of the bulb as mycelium or sclerotia and is planted with it in the fall. When the bulb starts into activity in the spring, the fungus starts also and sometimes spreads throughout the entire outer scale of the bulb. If the original infection is near the apex of the bulb, the shoot also is involved in the lesion and the mycelium growing from the bulb tissue infects the leaf tissue. This condition was frequently encountered in studying the disease and is well illustrated in figure 27. Usually it is only the outer, sheathing leaf that is diseased, although sometimes the whole shoot may be affected and fail to emerge from the soil. After growing in the leaf for a time, the mycelium emerges from the dead tissue and, if favorable conditions prevail, conidiophores and conidia are produced. These are formed on the aerial mycelium and also arise directly from the mycelium in the leaf. The unspecialized hyphae and the conidiophores which arise from the leaf emerge through the stomata, and in the specimens observed, only one came from each stoma.

Secondary inoculations and infections

The conidia, produced in great abundance on these first-infected leaves, furnish abundant inoculum for secondary inoculations. Although it is not improbable that they are also transported by such other agencies as insects, spattering rain, animals, and man, the conidia are for the most part scattered to the infection courts by means of the wind.

The infection courts may be any part of the tulip plant except the roots. Conidia falling on these parts germinate very quickly under proper conditions. Experiments with conidia in tulip juice and in distilled water produced successful germination. In the former there was a good development of germ tubes overnight, while in the latter germination took place but the development of the germ tubes was poor. This experiment was conducted at room temperature. The germ tubes penetrate directly, as discussed under the heading *Pathological histology* (page 351) and cause infection. Visible evidence of infection often appears within the short period of twenty-four hours, as was demonstrated in the pathogenicity experiments. Under conditions unfavorable for germination the conidia are able to retain their viability for some time, as the following experiment illustrates.

Tulip material abundantly covered with conidia was collected on June 12, 1917, at Ithaca, New York. It was kept under laboratory conditions and the capacity of the conidia for germination was tested on June 12, June 25, July 12, and August 2. Germination of conidia was obtained at all of these dates except the last. This shows that in a dry condition the conidia retain for several weeks their ability to germinate. The lesions caused by their infections soon enlarge and produce more conidiophores and conidia, which in turn are capable of producing more infections. That these infections are continually taking place is evident from the presence of lesions of various ages on the same leaf (fig. 25).

Conidia may be carried from badly diseased tops to the bulbs, perhaps being washed down by rain. Several specimens were collected which clearly showed this. Incipient lesions were found on both the stalks and the bulbs of such plants, showing how the inoculum works down to the bulbs. These lesions increase in size and sclerotia are produced. When the bulb becomes dormant the development of the lesion is arrested and the fungus is again ready for hibernation. There is no doubt that the sclerotia retain their vitality for a long period. In fact, isolations have been made from sclerotia which have been in a resting state for several years.

Pathological histology

The material was fixed in Flemming's, in chromo-acetic, and in Gilson's fluids, was embedded in paraffin, sectioned, and stained with both Heidenhain's iron alum-haematoxylon and Flemming's triple stain. Some difficulty was experienced in sectioning lesions on the bulbs because of the numerous, large, starch grains present. In order to study the

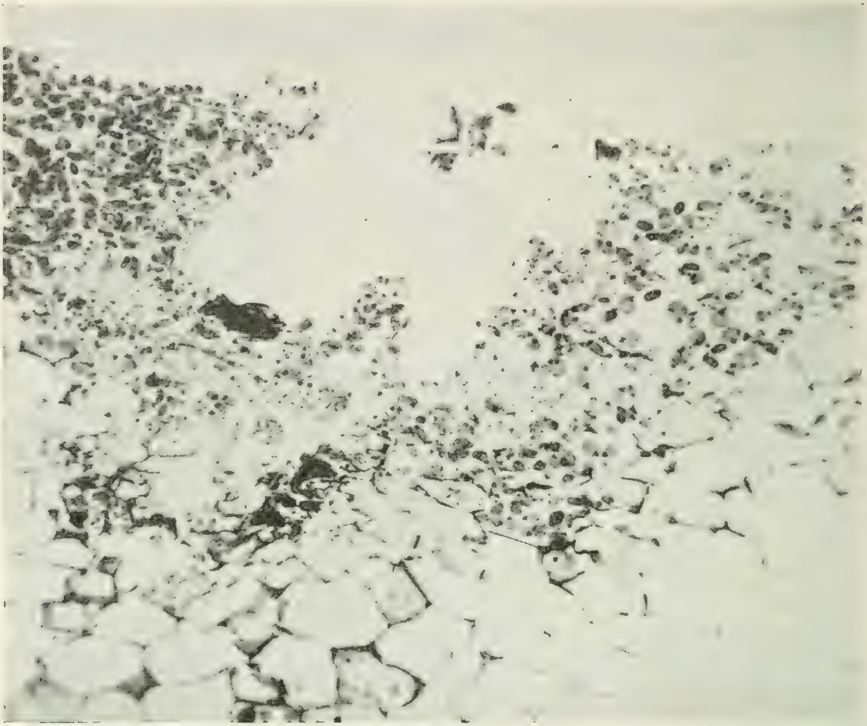

FIG. 43. LESION ON THE OUTER BULB SCALE

Photomicrograph of cross section through lesion. The accumulation of starch in the cells, and an incipient sclerotium, are visible

penetration, certain areas on the tulip leaves were marked with india ink, inoculated by dusting with conidia, and, after various intervals, cut out, killed, fixed, and stained.

On the bulbs, typical necrotic lesions appear, which show a peculiar accumulation of starch about the diseased area. This is pictured in its general features in figure 43, and in more detail in figures 44 and 45, one of which shows a diseased area and the other a healthy one. These starch grains are heart-shaped and large. They react to iodine in potassium iodide in the usual way, and with Flemming's triple stain are colored a beautiful pink. Why they should accumulate in this manner about the lesion is not known.

The mycelium in the bulbous tissue is usually of small diameter and is both inter- and intracellular. Usually in that part of the tissue where the mycelium is advancing and the cells are not yet killed, it is intercellular, while in the older part of the lesion the hyphae penetrate into the cells as well as between them. The protoplasm of the cells at this stage is practically gone. The collapse of these empty cells causes the lesion to be depressed. Sclerotia sometimes form on the surface of the lesion. In figure 43 an incipient sclerotium may be seen.

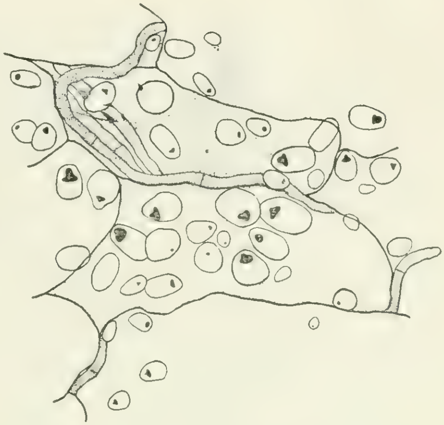

FIG. 44. STARCH ACCUMULATION IN DISEASED TISSUE. × 277

Starch cells in a diseased area of an outer bulb scale, showing numerous starch grains and intercellular mycelium. (Camera-lucida drawing)

Vascular bundles in the bulb scale were markedly affected, and in one case the xylem had entirely disappeared while the phloem, though attacked, still remained in part. In another specimen the bundle had been disintegrated on the side toward the lesion. This involved the phloem, the cells of which stained a deeper blue. There was starch accumulation in this region.

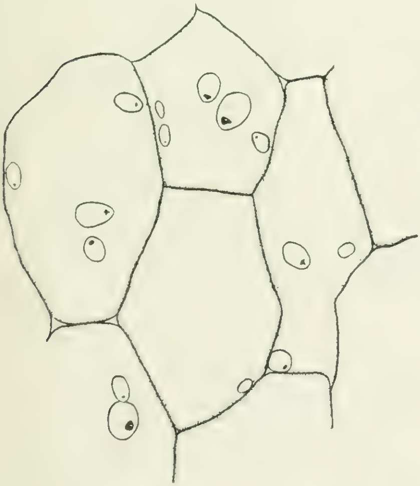

FIG. 45. HEALTHY BULB TISSUE. × 277

The amount of starch shown here may be compared with that shown in figure 44

Penetration of the fungus into the leaf tissue has been observed. No appressoria are formed by the germ tubes, which instead penetrate directly through the leaf surface, either through stomata or between epidermal cells (figs. 46, 47). The germ tubes have not been observed to penetrate directly through epidermal cells. It has been noted that penetration more often occurs where the conidia are more numerous. Probably this is because of the greater enzymatic action, which hydrolyzes the cuticular substance.

The mycelium in the leaf, like that in the bulb, is both inter- and intracellular. This is shown in figures 48 and 49. After the fungus has developed for a time in the leaf tissue, a collapse of the cells results and causes the leaf to become much thinner in the diseased area. Here also, where the mycelium is still intercellular, the cells are not killed. There is injury caused in advance of the mycelium. This indicates the excretion of toxic or enzymatic substances by the pathogene.

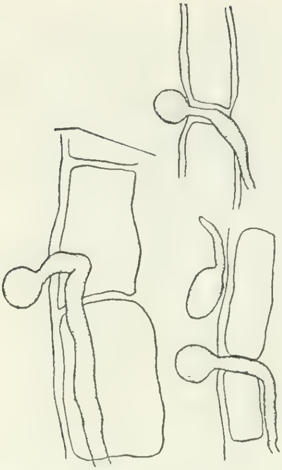

FIG. 46. PENETRATION OF LEAF TISSUE. × 277

Transverse section of epidermis. (Camera-lucida drawing)

The writer thinks that the injury caused in this disease is not due to oxalic acid. Some experiments were made to determine what the nature of the injury from oxalic acid would be. Several plants were injected hypodermically with solutions of oxalic acid of various concentrations, and lesions were produced which strikingly resembled those caused by a fungus. Furthermore, microscopical examination indicated that no fungus had been accidentally introduced. However, the concentrations were necessarily higher than those produced by fungi in culture. The work of Brown (1915) seems to show that neither oxalates nor oxalic acid take part in the toxicity of *B. cinerea*, but

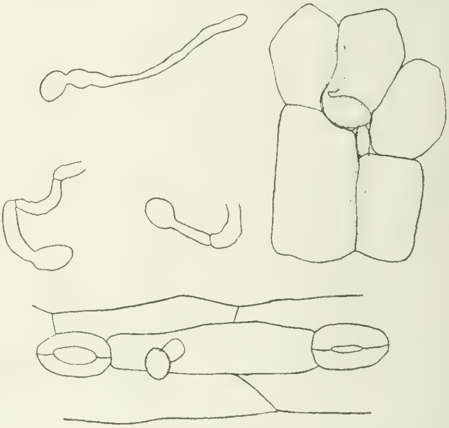

FIG. 47. PENETRATION OF LEAF TISSUE. × 277

Surface view. (Camera-lucida drawing)

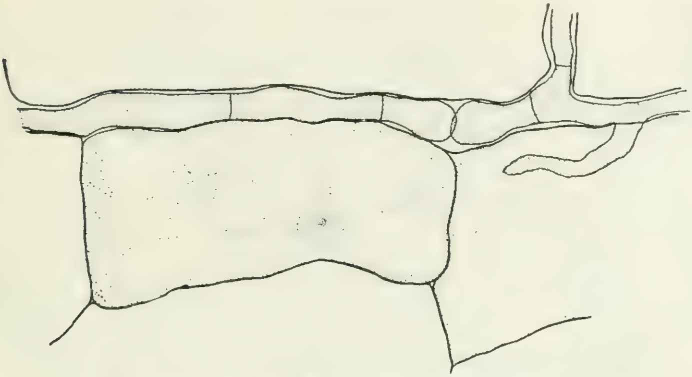

FIG. 48. INTERCELLULAR MYCELIUM IN LEAF TISSUE. × 600
The cells have not yet lost their protoplasm. (Camera-lucida drawing)

that this toxicity is due to enzymatic action.

CONTROL

It has been impossible to carry control experiments far enough to justify making any definite recommendations for the control of *Botrytis Tulipae.* A consideration of the pathogene, however, makes it evident that elimination is probably of first importance. Clean bulbs, free from mycelium and sclerotia, should produce clean tulips, for it is most probable that in these forms the pathogene is carried on the bulbs. Although the disease may possibly be attributed to infested soil, it often occurs on tulips grown in soil in which heretofore no tulips have been grown.

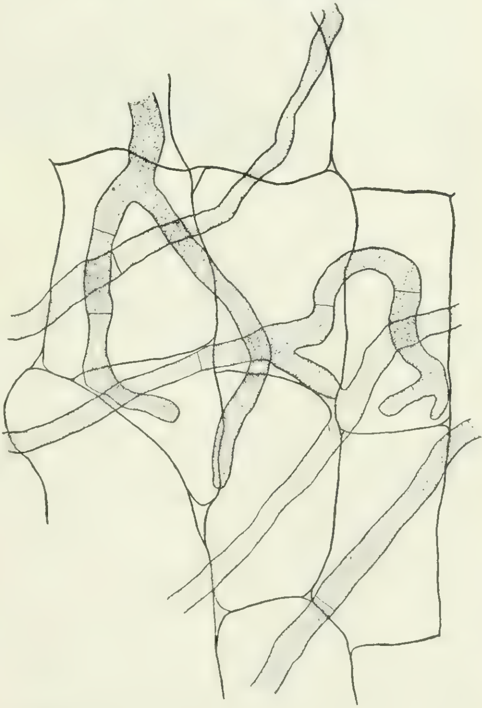

FIG. 49. INTRACELLULAR MYCELIUM IN LEAF TISSUE. × 600
The cells of the leaf are devoid of contents. (Camera-lucida drawing)

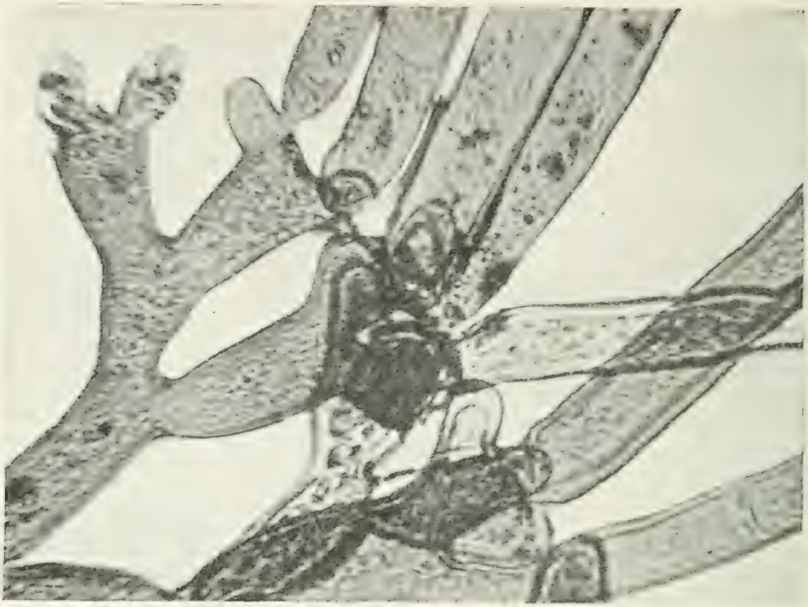

FIG. 50. PROLIFERATION OF CONIDIOPHORE. × 840
Showing new branches arising from conidial cluster. (Photomicrograph)

Until further experiments have been made, soil treatment cannot be recommended. Carbolineum has been recommended in the literature as a disinfectant for soil (Elenkin, 1911). Klebahn (1904:33) criticizes this method unfavorably, saying that not even weeds will grow in soil thus treated.

Experiments made at Madison, Wisconsin, in 1917, showed that spraying tulips with bordeaux mixture 5–5–50 caused considerable injury to both the leaves and the flowers, besides giving them an unsightly appearance. Accordingly this treatment is not to be recommended.

From the present knowledge of this disease the following measures seem advisable:

1. Selection of clean bulbs, free from lesions and sclerotia. When the sclerotia occur only on the outer papery scale this should be removed and burned. It is well also to inspect the old stalk of the previous year, if this still remains attached, for it frequently bears sclerotia (fig. 22).

2. Careful handling, to avoid injuring the bulbs, as infection takes place more readily on injured bulbs than on healthy ones.

3. Storage of the bulbs under proper conditions of temperature and humidity. The temperature should be kept as low as possible without injury to the bulbs, preferably about 40° F. The humidity also should be low. These conditions are especially desirable, as they retard the development of any small lesions that may be present on the bulbs at the time of storage, and prevent the germination of any conidia that may be on their surface.

4. Removal and destruction of diseased plants when they appear in the field or the beds. This will limit, if it does not entirely prevent, secondary infections.

SUMMARY

An investigation of the tulip disease caused by *Botrytis Tulipae* (Libert) comb. nov. shows that it is present throughout the United States and that it was probably introduced with the introduction of tulip bulbs. Reports of the disease show that it has been in this country at least since 1901.

Under normal conditions this disease is restricted to the genus Tulipa and within this genus practically all varieties are susceptible. One instance of apparent immunity is the variety Baronne de la Tonnaye, which, during an epidemic of Botrytis blight, showed no evidence of the disease.

Counts made in the spring of 1917, at Ithaca, on one variety of late tulips, Spathulata, showed 100 per cent of the leaves and 98 per cent of the stalks to be affected. Other varieties were similarly infected. These infections were traced to the bulbs, of which the variety Spathulata showed 4.6 per cent with unmistakable Botrytis lesions and the Mrs. Grover Cleveland variety, 5.2 per cent.

The disease is easily recognized on the bulbs when the fungous sclerotia are present in the lesions. On the leaves, the flower stalks, and the flowers, a severe blighting frequently takes place.

Studies of the literature and herbarium specimens show that the disease under consideration is to be ascribed to *Botrytis Tulipae* (Libert) comb. nov.[4]

Cultural studies have demonstrated that both the small sclerotia on the bulbs and the conidial form on the leaves and other parts of the tulip plant are stages of one and the same fungus, namely, *Botrytis Tulipae.*

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[4] In recent literature the fungus has gone by the name *Botrytis parasitica* Cavara, but the specific name of Libert has priority.

The morphology of the parasite has been investigated in some detail. The manner of formation and attachment of the conidia has been brought out, microconidia have been demonstrated for this species and conidial measurements show that while the variation in size is from 12–24 x 10–20, the greater number of spores measure 16–17 x 9–10.

Conidial production, which rarely takes place in pure cultures under ordinary conditions, was found to occur abundantly when plate cultures were partially dried. Abundant conidia were also formed on sterilized tulip leaves in large test tubes.

The parasitism of *B. Tulipae* has been fully demonstrated by numerous infections brought about by the use of pure cultures of the organism. Inoculations of other plants, both nearly and distantly related, while showing the parasite to be restricted to tulips, show also that the parasite exhibits a weak and varying degree of ability to attack other plants.

Hibernation is by means of sclerotia which live over the winter on the bulbs. Infection spreads from these bulbs to the developing shoots, where abundant conidia are produced. These primary lesions serve as the source of inoculum for secondary infection. The conidia produced in this manner retain their vitality for several weeks.

Sections through lesions on the bulb show an accumulation of starch about the diseased area. In the penetration of the tissue by conidial germ tubes, no appressoria are formed and the germ tubes penetrate directly through the epidermis or through the stomata.

Although extensive control experiments have not been made, it is recommended that clean bulbs, careful handling of bulbs, proper storage, and systematic removal and destruction of diseased plants in the field will largely hold the disease in check.

Memoir 39, *The Genetic Relations of Plant Colors in Maize*, the sixth preceding number in this series of publications, was mailed on July 19, 1921.

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CROWN-GALL OF APPLE AND PEACH WITH NOTES ON THE BIOLOGY OF BACTERIUM TUMEFACIENS

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CROWN-GALL OF APPLE AND PEACH WITH NOTES ON THE BIOLOGY OF BACTERIUM TUMEFACIENS

DONALD REDDICK AND V. B. STEWART

THE CROWN-GALL DISEASE

The crown-gall disease, caused by *Bacterium tumefaciens* Smith & Townsend,[1] occurs on a great variety of plants and over a wide geographical range. It has been noted particularly in fruit culture from practically every fruit district in the United States, and the very general opinion expressed in the literature on this subject is that the disease is a dangerous one and its introduction into a planting is greatly to be feared.

The evidence bearing on the economic importance of the disease is largely observational and few experimental data are at hand to show its exact importance. The observational data are fully summarized by Smith, Brown, and Townsend[2] and need not be repeated here. In general, it may be concluded from these authors' digest of the literature that apples are not seriously affected but that peaches and many other plants are. These writers are inclined, however, to include apples (page 197 of the bulletin cited) in the list of plants that should be inspected for crown-gall, and to recommend rejection of trees showing galls or hairy root. They suggest that even if apple trees are not materially affected by crown-gall, the distribution of trees bearing galls will serve to spread the disease to new localities and to other hosts which are more susceptible and which may be injured thereby. Neither point seems to be well taken when one considers (1) the very wide distribution of the disease at the present time, and (2) that the apple constitutes a relatively permanent crop which is not interplanted except for a few years and then preferably with cultivated crops such as potatoes, beans, corn, and the like, that is, plants which do not develop the disease under natural conditions.

In a more recent publication, Erwin F. Smith[3] states that "when death results it is not due to the direct action of the bacteria, but to other factors, *e.g.*, nutritional defects, and secondary parasitisms," and it is to be expected, therefore, that climatic conditions, soil conditions, and various other factors may enter to cause even greater variations in different regions than would result if only the one complication were involved. For this reason, regional tests must be made to determine the destructiveness of the disease for any particular set of conditions.

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[1] Attention is called to the fact that this organism is named *Bacterium tumefaciens* in conformity with the nomenclature of Erwin Smith, which is in common use among plant pathologists. According to the nomenclature commonly followed by bacteriologists, however (see *Bergey's Manual of Determinative Bacteriology*, 1923), this organism would be called *Phytomonas tumefaciens*.

[2] Smith, Erwin F., Brown, Nellie A., and Townsend, C. O. Crown-gall of plants: its cause and remedy. U. S. Dept. Agr., Bur. Plant Ind. Bul. 213:1–215. 1911.

[3] Smith, Erwin F. Further evidence that crown gall of plants is cancer. Science 43:871–889. 1916. (The quotation cited is on page 876.)

In this connection it is to be recalled that some years ago F. C. Stewart [4] stated before a convention of fruit growers that "in New York, at least, apple crown gall is an unimportant disease." Stewart cites the C. H. Stuart orchard at Newark, New York, as an example. It has been objected that C. H. Stuart was engaged in the nursery business at the time when the orchard was set (the inference being that he was therefore not disinterested), and that apparently no healthy trees were set for comparison. The former fact practically assures that the trees really had crown-gall disease, and the latter is a natural mistake sometimes made by others than merely practical men.

The controversy concerning the number of trees originally set in the Stuart orchard, and particularly the question as to whether one hundred of the original trees died, possibly from crown-gall, led one of the writers (R) to visit the orchard. The following facts were either ascertained from E. V. Pierson (the present owner and a son-in-law of C. H. Stuart), or determined by personal observation. The orchard is one and a half miles directly north of Newark on Main Street. It is on the right-hand side and a little back from the road, and is permanently marked by a large stone some three feet in diameter which lies at the southwest corner. The exact limitations of the original planting of galled trees cannot be determined. There are more than a thousand trees in the orchard, all apparently of the same age and of the same general appearance. The block, therefore, constitutes a satisfactory experiment, because at least as many gall-free trees were used as trees bearing galls. The number of galled trees planted originally is of little consequence, but Mr. Stuart's statement to F. C. Stewart may be accepted as the more likely number, namely, 500 trees. The galled trees in particular constitute a mixed lot of varieties. Baldwin, Greening, Sutton, and Ben Davis were recognized, and others are present. The remainder of the orchard seems to be somewhat mixed, also. The experiment indicates that, to date, crown-gall has done no material damage. Allowing for the numerous varieties set indiscriminately, the orchard is pronounced "average" by W. H. Chandler, Professor of Pomology at Cornell University. Trees have died here and there and have been replaced. Excepting for a wet area which scarcely could have been included in the block of galled trees, the number of missing trees is not greater in one area than in another.

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[4] Stewart, F. C. Interesting facts about crown gall of apple and peach. West. New York Hort. Soc. Proc. 53:97–98. 1908.

Recently, experimental data have been published on crown-gall experiments with apples, from the States of Iowa[5] and Montana.[6] The experiments were planned and executed on a large scale. In Montana it appears that while there was no noticeable effect on the vigor of the trees, nevertheless the root development of galled trees was not good, and it is thought that this would have become apparent when the trees began to bear heavy crops. In Iowa numerous growth data were recorded, and these show without exception that galled trees did not grow so fast as did trees free from gall. It should be noted, in this connection, that the method employed was one which has not yet received general indorsement by horticulturists.

An objection to all the experiments reported, including the one about to be recorded, is that the real test comes only with the production of fruit (because the trees are grown for the fruit they bear, not for the wood they produce). A well-planned experiment to determine the effect of crown-gall should extend well into or through the life of the plant. This would be a very long time for apple trees, but could be done relatively easily with peaches.

A proper interpretation of any of the crown-gall experiments thus far reported is rendered difficult by the fact that the experimental trees used have consisted of a miscellaneous collection for which there are no data as to original source, particularly as to the exact character and origin of the stock on which the trees were grown. The Iowa experiments illustrate this very well. One of the conclusions of Greene and Melhus is that Wealthy is more susceptible to crown-gall than is Jonathan. It is difficult to understand how this can be, since the varieties were grafted on seedling stock and an examination of the illustrations indicates that the galls are confined almost entirely to the stocks. It thus becomes necessary to assume an influence of scion upon stock which is not yet admitted by horticulturists. Under the circumstances it may be assumed with equal probability that the conditions of the experiment were such that a large variable factor was not brought under control. The Iowa experiment is further complicated by the fact that the plot of land in which the trees were planted had grown nursery stock some of which was affected with crown-gall, and that the plot is so located that it, or at least a part of it, received the wash from adjacent affected trees. Furthermore, the report does not state whether the trees were planted in such a way as to eliminate variations in soil and in growth conditions. It must be remembered in this connection that in average orchard planting the chance of infection after the trees are permanently set is very small.

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[5] Greene, Laurenz, and Melhus, I. E. The effect of crown gall upon a young apple orchard. Iowa Agr. Exp. Sta. Research bul. 50:145–176. 1919.

[6] Swingle, D. B., and Morris, H. E. Crown-gall injury in the orchard. Montana Agr. Exp. Sta. Bul. 121:123–139. 1918.

The crown-gall disease seems to have been much more prevalent in New York some fifteen or twenty years ago than it is at the present time. There was much more agitation about the disease at that time, but this may be accounted for in part by the fact that inspection service for San José scale was being instituted and enforced, and that this inspection involved also examination for and rejection of trees having crown-gall. Aside from this, however, one of the writers (S), who was interested in diseases of nursery trees, recalls that in 1909 the fields in one large nursery from which apple trees had been dug were thickly strewn with trees that had been rejected at digging time because of crown-gall.[7] It was this condition, in part, that led to the experiments herein reported. But in 1910, when trees were sought for the experiments, very few were to be found and there was some difficulty in procuring the necessary stock. This condition has prevailed in subsequent years. One factor in nursery management may have contributed to this desirable condition, namely, that there has been an increasing tendency on the part of the nurserymen to grow trees in rotation with farm crops, thus allowing for the death of *Bacterium tumefaciens* in the soil or on plant parts. Another factor which may be involved is that most apple trees in New York are budded on French stock. One of the writers (S)[8] has expressed the opinion that French-grown stock is less susceptible to crown-gall than is American-grown stock, and it seems obvious that the practice of budding in the field, which is the common practice in New York, gives less opportunity for widespread infection than does that of bench grafting.

PLAN OF THE CROWN-GALL EXPERIMENT

In order to determine, if possible, the importance of crown-gall on apple and peach trees under the conditions existing at Ithaca, New York, an experiment was planned, the details of which were as follows:

The land for the experiment lay in a low field which had been in grass for a number of years previously. There are hills on all sides, but there is suitable drainage so that the soil is never too wet for good growth. The soil is a good loam, running out into stony loam on the side where the rows are marked *A* in figure 1. The soil is suitable for the growth of both apples and peaches, but the location is unsuited for peaches. Peaches are grown successfully a few miles from Ithaca, but there is little chance of securing crops in the immediate vicinity, chiefly because of low temperature in the winter. This was not fully realized at the beginning of the experiment.

The land was ploughed and cultivated each year and a sown cover crop usually was employed.

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[7] It is likely, though, that some of these trees bore galls produced by an aphis (*Schizoneura lanigera*), and this is the opinion expressed by one large nurseryman.

[8] Stewart, V. B. Exclusion legislation and fruit tree production. Phytopath. 8: 360–364. 1918. (The citation refers to page 362.)

THE EXPERIMENT WITH APPLES

Apple trees, of several varieties, bearing evident lesions of crown-gall were procured in the autumn of 1910. On April 20, 1911, photographs were made of the trees[9] and the trees were set in the garden. On May 6, healthy trees were procured, photographs were made, and the trees were interspersed with the diseased trees as shown in the accompanying plan (figure 1). All the trees were two years from the bud and were on imported French stock. Practically all of them, except for the galls, would have been graded as best-quality trees. The notable exception is tree C-6, variety Alexander, which was small in diameter and bore a very large gall at the crown.

	1	2	3	4	5	6	7	8	9
A	**S**	**We**	S	**We**	**S**	We	**S**	**We**	**S**
B	**We**	**TO**	**We**	TO	**We**	**TO**	Wa	**TO**	**Wa**
C	**B**	**Wa**	**B**	**Wa**	B	**A**	**B**	**Wa**	**K**
D	**Wa**	**WR**	**Wa**	**WR**	**Wa**	**K**	WR	**K**	K

FIGURE 1. PLAN OF THE APPLE CROWN-GALL EXPERIMENT

Each character represents a tree and indicates the name of the variety according to the following key: *A*, Alexander; *B*, Baldwin; *K*, Tompkins King; *S*, Northern Spy; *TO*, Twenty Ounce; *Wa*, Wagner; *We*, Wealthy; *WR*, Wolf River. Bold-face letters represent galled trees; light-face letters represent healthy trees

The trees were pruned very lightly from year to year, and in all cases the pruning consisted in the entire removal of undesirable limbs or branches. No heading-back was done.

A record of the general appearance of the trees was made twice each year, always without notes of the previous condition. For the most part the records are normal and may be tabulated without comment. One tree, C-3, was girdled by mice in the winter of 1913-14. A renewal developed but was winterkilled the following winter. The tree was removed on May 28, 1915, when it was found that the galls had enlarged appreciably (figure 2). The record of this tree previous to injury is shown in table 1.

The records for the apple trees are given in table 1. Before examining the records or deriving any conclusion, the reader should have in mind a number of items. (1) It is now evident that the experiment was not planned on a large enough scale to give the best results, and particularly there were not enough healthy trees planted for comparison. This was not an oversight on the part of those planning the experiment, but rather was due to space limitations. (2) The varieties employed in the test were so numerous and the growth habits so different that comparisons between varieties would lead to error. On the other hand, the number of trees of any one variety was scarcely large enough to allow for satisfactory comparisons. (3) The yield of the trees was expected to give quantitative data. The trees had only just come into bearing, and so these data were not obtainable at the time when, of necessity, the experiment was terminated. It is not likely that the data would have been of sufficient extent to be very reliable. (4) The apple-tree borer (*Saperda candida*) proved a great pest. Sometimes as many as six larvae were found in a single tree. If these larvae are removed, little injury will follow; but if one or two are missed—and this apparently is almost inevitable—great damage may be wrought within a few weeks. Most of the fluctuations in general appearance of trees from year to year, without question, are attributable to this borer. (5) Frost, rabbits, mice, and other fortuitous circumstances reduced the size of some trees so that, with the small numbers involved, it did not seem worth while at the end even to weigh the trunks and the crowns, although this could have been done very easily.

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[9] The photographs are on file in the Department of Plant Pathology at Cornell University under the number 5727. None is reproduced here because the negatives are uniformly poor and suitable reproductions cannot be made. Camera and plates were tested in order that uniformly good photographs might be obtained without waiting to develop the negatives. When all negatives were found to be uniformly bad, it appeared probable that the shutter of the camera had been tampered with in the interim. The original negatives show the location and size of the galls very readily, and they proved most useful when the trees finally were removed.

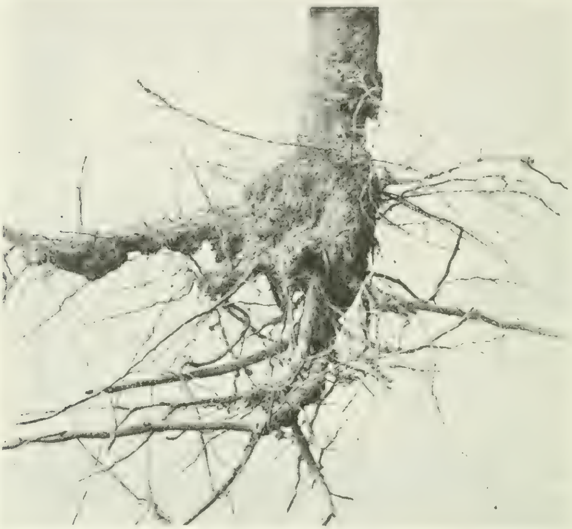

FIGURE 2. BALDWIN TREE C-3

The galls had increased somewhat in size, but there was no indication that they had interfered with the development of the tree

TABLE 1. ANNUAL RECORD OF GROWTH AND DEVELOPMENT OF HEALTHY APPLE TREES AND OF TREES AFFECTED WITH CROWN-GALL

(The trees were set in the spring of 1911 and records were made from 1912 to 1919 inclusive. The final record was made on May 20, 1920. In examining the trees for general condition and appearance, four classes were recognized: *e*, excellent; *g*, good; *f*, fair; *p*, poor. Healthy trees are designated by an asterisk)

Variety	Location	General condition of growth for the respective seasons								Condition of roots at digging time
		1912	1913	1914	1915	1916	1917	1918	1919	
Northern Spy..	A-1	p	p	f	g	f	f	f	e	g. No gall
Northern Spy..	*A-3	f	g	e	e	e	e	e	e	e. No gall
Northern Spy..	A-5	g	g	e	e	e	e	e	e	e. No gall
Northern Spy..	A-7	f	g	e	e	e	e	e	e	f. Hard gall at crown, size of a liter flask
Northern Spy..	A-9	g	g	e	e	e	e	e	e	g. No gall
Wealthy.......	A-2	f	g	g	g	g	g	g	e	g. Small galls on one large root
Wealthy.......	A-4	g	g	e	e	e	e	e	e	g. Two small hard galls at crown; small galls on two roots with hairy root
Wealthy.......	*A-6	f	g	e	e	e	e	e	e	g. No gall
Wealthy.......	A-8	g	g	e	e	e	e	e	e	e. No gall
Wealthy.......	B-1	e	g	g	g	g	g	g	e	e. No gall
Wealthy.......	B-3	e	g	e	g	...[1]	....	....	....	....
Wealthy.......	B-5	f	f	f	f	f	f	f	f	e. One hard gall (egg) on trunk; smaller galls on several roots
Twenty Ounce..	B-2	e	e	e	e	e	e	e	e	e. No gall
Twenty Ounce..	*B-4	f	g	g	e	e	e	e	e	e. No gall
Twenty Ounce..	B-6	e	g	e	e	e	e	e	e	Broken off at crown
Twenty Ounce..	B-8	g	g	....	e	e	e	e	e	g. No gall
Wagner........	*B-7	f	g	e	e	e	e	e	e	g. No gall
Wagner........	B-9	f	g	....	g	e	g	e	e	g. No gall
Wagner........	C-2	g	g	e	e	e	e	e	e	e. No gall
Wagner........	C-4	f	g	e	g	g	g	g	e	g. Remnant of hard gall in crown; small galls (egg) on two roots
Wagner........	C-8	g	g	f	f	g	f	g	f	g. Hard gall (egg) in crown and on one root; small gall on one root
Wagner........	D-1	g	g	g	e	e	e	e	e	g. No gall
Wagner........	D-3	e	e	e	e	e	e	e	e	g. No gall
Wagner........	D-5	g	g	e	e	e	e	e	e	g. One small (metastatic?), recent, hard gall on trunk above ground
Baldwin.......	C-1	g	g	e	e	e	e	e	e	e. No gall
Baldwin.......	C-3	g	g	...[2]	....	....	....	....	....	....
Baldwin.......	*C-5	e	e	e	e	e	e	e	e	g. No gall

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[1] Girdled by borer; removed October 11, 1916; gall in crown 8 centimeters in diameter.

[2] Girdled by mice; removed May 28, 1915; gall enlarged. (See figure 2.)

TABLE 1 (*concluded*)

Variety	Location	General condition of growth for the respective seasons								Condition of roots at digging time
		1912	1913	1914	1915	1916	1917	1918	1919	
Baldwin	C-7	g	g	g	g	g	g	g	f	g. No gall
King	C-9	e	g	e	e	e	e	e	e	g. Hard gall (egg) at surface of ground
King	D-6	g	e	e	e	e	e	e	e	g. No gall
King	D-8	e	g	e	e	e	e	e	e	e. No gall
King	*D-9	f	g	e	e	e	e	e	e	g. No gall
Wolf River	D-2	e	e	e	e	e	e	e	e	e. Doubtful galls on a few small roots
Wolf River	D-4	e	e	e	e	e	e	e	e	e. Small gall (egg) in crown
Wolf River	*D-7	e	e	e	e	e	e	e	e	e. No gall; stock enlarged (natural?)
Alexander	C-6	e	e	e	e	e	e	e	e	e. Stock enlarged on one side; hard gall present

The trees were removed on May 24, 1920. About two-thirds of them were pulled by hitching a team directly to them; the remainder were pulled with blocks. There was not the least indication that gall-free trees pulled more easily than those originally bearing galls. One tree, B-6, broke off at the surface of the ground, but this seems to have been due to weakening from borers and to the large size of the roots.

On examining the roots it could not be detected, from their appearance, size, or length, that there was any difference between the roots of galled trees and those of trees free from galls. The most surprising thing revealed by examination of the roots was that in many instances the original galls had disappeared completely. This was true in practically all cases in which the original tree had a long rootstock and the gall was located near the base of the stock. All the main roots issued from the stock near the surface of the ground, due perhaps to the nature of the subsoil. In most cases in which the gall was located on a lateral root, both root and gall had disappeared, but the same disappearance of original roots was noted in healthy trees also.

Summary for the apple experiment

The data in table 1 may be summarized in a few words. In no case is there indication that the presence of crown-gall on the roots of these apple trees interfered in any way with growth. The galls had largely disappeared, and judging by this test the crown-gall disease on apples seems, in this locality, to be chiefly a matter of concern to the nurseryman.

Even in the nursery it is not certain that the disease is of great consequence for apples. It will be noted that most of the trees used in the test were of a size to be graded first-class. Galled nursery trees have been seen that were short and very small in diameter, and the natural inference is that the presence of the disease accounted for the size of the tree. It is to be remembered, however, that trees of small caliper are not uncommon in the nursery even when no disease is present. Tree C-6 of the experiment is of this sort, and its behaviour when planted in the orchard is of particular interest. Two companion trees of C-6 were rejected for the experiment because their dwarfed condition obviously made them unfair to the test. One of the writers (R) planted these two trees in deep sandy loam on private land. Both trees have made excellent growth, and as they were top-worked to early-maturing varieties both have borne crops of fruit. One tree was removed in 1922 to make way for a building. There was no evidence of the original gall or of subsequent infection. The other tree is larger than some others that were set in the immediate vicinity two years earlier.

In connection with the outcome of this experiment the experience of a well-known horticulturist is of interest. The following extract is taken from an article by C. A. Green, for many years editor of *Green's Fruit Grower*. The article appeared in the issue of that journal for May, 1912. The orchard mentioned in the article is located twelve miles southwest of Rochester, New York, and is of special interest because it is in the main apple belt of western New York.

> Here is something that I want to call particular attention to. There is much said in these days about root gall on apple trees. At the time I planted this orchard but little was known about root gall. As I planted the trees with my own hands I noticed that occasionally a tree had a knotty excrescence on the roots which I now would recognize as root gall, and which is now regarded as infectious and fatal to the growth or welfare of the trees. Here is an interesting point which I desire to allude to: after growing for over thirty years and bearing fruit abundantly for over fifteen years, every tree in this orchard is healthy and one tree is as productive as another, and yet a number of these trees were infested with root gall. If the views of the experiment station are correct, the trees I planted thirty years ago, which were then affected with root gall, should long ago have perished or should have shown some signs of disease. That the trees are now all healthy and productive, teaches me that we have something yet to learn about root gall on apple trees.

THE EXPERIMENT WITH PEACHES

In the spring of 1911 a quantity of peach trees bearing evident lesions of crown-gall were procured from New York nurserymen. On April 6, 1911, photographs were made and the trees were set in the diseased garden as indicated in figure 3. Healthy trees were obtained in a separate shipment. The healthy trees were prepared for planting by one man, who used all antiseptic precautions. They were planted before the general lot of infected trees, in order to avoid any contamination. All the trees were one year from the bud and were on peach stock. They were interplanted in the block of apples so that the whole block was on the quincunx plan with trees ten feet apart in the rows.

	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17		18	
A	**B**		**B**		**B**		**B**		B		B		**B**		**B**		**B**		**B**		**B**		**B**		**B**		**B**		**B**		**B**		**B**		**B**	
B		**B**				**B**				**B**				**B**				**B**				**B**				**B**				**B**				**B**		**B**
C	**B**		**B**		**B**		**B**		C		C		**C**		**C**		**C**		**C**		**C**		**C**		**C**		**E**		**C**		**C**		**C**		**C**	
D		**C**				**C**				**C**				**C**				**C**				**E**				**C**				**C**				**C**		**C**
E	**C**		**C**		**C**		**C**		**C**		**C**		**C**		**C**		**C**		**C**		C		C		**C**		**C**		**C**		**C**		**C**		**C**	
F		**E**				**E**				**E**				**E**				**E**				E				E				**E**				**E**		**E**
G	**E**		**E**		**E**		**E**		**E**		**E**		**E**		**E**		**E**		**E**		E		E		**E**		**E**		**E**		**E**		**E**		**E**	
H		**F**				**F**				**F**				**F**				**F**				F				F				**F**				**F**		**B**

FIGURE 3. PLAN OF THE PEACH CROWN-GALL EXPERIMENT

Each character represents a tree and indicates the name of the variety according to the following key: *B*, Belle of Georgia; *C*, Carman; *E*, Elberta; *F*, Foster. Bold-face letters represent galled trees; light-face letters represent healthy trees

A great many of the trees failed to start growth when set, with the result that twenty-eight of the original ninety-eight affected trees were removed the first year. All of the affected trees of the variety Foster succumbed early. This may not be significant, because the original source and the treatment of these trees are not definitely known. From the high percentage of failures the first summer, it would appear that the crown-gall disease may be a factor in securing a good stand. This could be determined readily, but, unfortunately for the experiment, the crown-gall disease has been very scarce on peaches as well as on apples in New York nurseries since 1910, and doubtless for the same reason.

The winter of 1911-12 was severe, and winter sun-scald was abundant not only in the experimental block but throughout western New York. The loss from winter injury might be ascribed conveniently to a weakened condition of the trees due to the presence of crown-gall. The record shows, however, that 53 per cent of the healthy trees showed this injury, whereas only 20 per cent of the diseased trees were injured in this way. Furthermore, the winter of 1910-11 also was exceedingly severe for fruit trees of all kinds. Nurserymen generally were called on to replace many of the trees set in the spring of 1911. One firm alone replaced 20,000 peach trees out of a season's business of perhaps 300,000 trees. This may mean that two or three times the number replaced actually died, since, as a rule, not many persons demand replacement. For the most part the injury to the experimental trees occurred above the snow line, so that new growth started below the frost canker and above the point of union of scion with stock. Even when the renewal came from below the union, the tree was allowed to stand, since the development of the galls should be approximately the same.

In subsequent years many of the trees made excellent growth, as is shown in table 2, but each winter the temperature was low enough to kill fruit buds or else the buds were forced by a period of warm weather in late winter and then killed by frost.

The larvae of a borer (*Sanninoidea exitiosa*) gained entrance to a number of the trees in the late summer of 1913. As they were not noted until the following year, the number of trees showing effects of borer injury in 1914 is relatively large. The foliage of affected trees was slightly yellow (off color), was often somewhat rolled upward, and in some cases showed a marked tinge of red on the margins. Some of the borer channels extended far into the roots, and practically all of the trees removed on September 1, 1915, were injured primarily by borers. In some cases frost cankers were present, but these probably followed as a result of poor growth or immature wood consequent to borer injury.

Results of the peach experiment

From time to time the trees that were crowding, and especially those that were in poor condition, were removed. It was thus determined that borers were chiefly blamable for the poor appearance of certain trees, and that for the most part the galls had disappeared entirely. In many cases the position of the original gall could be determined only with difficulty or not at all. Usually the position could be determined by the occurrence on the stock of an area having the appearance of a healed wound (Plate II).

No crop of fruit was obtained during the course of the experiment, and so the only record is on the general appearance of the trees and the condition of the roots at digging time. The condition of the trees from year to year is shown in table 2. The same general reservations mentioned for the apples (page 7) apply in the case of the peaches, although the exact reason for the reservation is not the same in every instance.

The trees still living in the spring of 1918 were pulled. Unfortunately they were removed without warning and were not left in their regular order. An attempt was made to compare the leafless trees with previous growth records, but there was so much uncertainty about this that it was abandoned. Examinations of the pulled trees bore out the condition indicated in the table, namely, that most of the galls had disappeared.

The most interesting outcome of the experiment is that no peach trees died from the effects of crown-gall, and, so far as can be determined, the disease did not affect the growth of the trees to any appreciable extent. For the most part, galls either disappeared or were relatively small and insignificant. In a few cases the whole stock of the tree seems to have been enlarged, but even in such cases there was no indication, from the general appearance and growth of the tree, that any injury had been done.

TABLE 2. ANNUAL RECORD OF GROWTH AND DEVELOPMENT OF HEALTHY PEACH TREES AND OF TREES AFFECTED WITH CROWN-GALL

(The trees were set in the spring of 1911 and records were made from 1912 to 1917 inclusive. The final record was made on May 20, 1918. In examining the trees for general condition and appearance, four classes were recognized: *e*, excellent; *g*, good; *f*, fair; *p*, poor. Healthy trees are designated by an asterisk)

Variety	Location	1911 Oct. 3	1912 Oct. 12	1913 Sept. 6	1914 Sept. 14	1915 Sept. 14	1916 Sept. 11	1917 Sept. 25
Belle of Georgia	A- 1	.....	.....[1]	.....	.....	.....	.....	....
Belle of Georgia	A- 2	.....	f	f	g	g	g	f
Belle of Georgia	A- 3	Dead[2]	.....	.....	.....	.....	.....	......
Belle of Georgia	A- 4	.....	g	g	f	p[4]	.....	......
Belle of Georgia	*A- 5	.....	e	g	g	g	e	e
Belle of Georgia	*A- 6	.....	e	g	g	g	e	e
Belle of Georgia	A- 7	.....	e[3]	g	g	g	e	e
Belle of Georgia	A- 8	.....	e	g	g	g	e	e
Belle of Georgia	A- 9	.....	e	g	g	g	e	e
Belle of Georgia	A-10	.....	e[3]	g	g	g	e	e
Belle of Georgia	A-11	.....	f	g	g	g	g[5]	......
Belle of Georgia	A-12	Dead[2]	.....	.....	.....	.....	.....	......
Belle of Georgia	A-13	.....	g	g	g	g	e	e
Belle of Georgia	A-14	.....	g	g	g	g	e	e
Belle of Georgia	A-15	.....	g	g	g	g	g	g
Belle of Georgia	A-16	.....	g[3]	g	g	g	f[6]	......
Belle of Georgia	A-17	Dead[2]	.....	.....	.....	.....	.....	......
Belle of Georgia	A-18	.....	e[3]	f	f	f[7]	.....	......
Belle of Georgia	B- 1	.....	e[3]	e	g	g	g[8]	......
Belle of Georgia	B- 2	.....	e	e	e	e	e	e
Belle of Georgia	*B- 3	.....	e	g	g	e	e	g
Belle of Georgia	*B- 4	.....	e	g	e	e	e[9]	......
Belle of Georgia	B- 5	.....	e[3]	g	e	e	e	e
Belle of Georgia	B- 6	Dead[2]	.....	.....	.....	.....	.....	......
Belle of Georgia	B- 7	.....	g	g	e	g	g	e
Belle of Georgia	B- 8	Dead[1]	.....	.....	.....	.....	.....	......
Belle of Georgia	B- 9	.....	e	g	e	g	e	e
Belle of Georgia	B-10	Dead[2]	.....	.....	.....	.....	.....	......
Belle of Georgia	H-10	.....	e	e	e	f	g[10]	......
Belle of Georgia	C- 1	.....	....[1]	.....	.....	.....	.....	......
Belle of Georgia	C- 2	.....	e	g	g	g	g	f
Belle of Georgia	C- 3	.....	e	g	e	e	g	g
Belle of Georgia	C- 4	.....	e	g	g	e	e	e
Carman	*C- 5	.....	e	g	g	e	e	g
Carman	C- 7	Dead[2]	.....	.....	.....	.....	.....	......
Carman	C- 8	.....	p[3]	g	g	f[11]	.....	......

---

[1] Did not survive the winter.

[2] Failed to grow, or a few weak shoots pushed and then died.

[3] Girdled by frost; a new shoot developed from below the snow line.

[4] Removed September 1, 1915; winter injury; gall inactive, small.

[5] Removed October 11, 1916; crowding; good roots; crown considerably enlarged; several small galls.

[6] Removed October 11, 1916; roots fair; one small gall in crown; severe borer injury.

[7] Removed September 1, 1915; winter injury; gall disappeared.

[8] Removed October 11, 1916; excellent roots; large galls on crown; one gall 2 centimeters in diameter on one root.

[9] Removed September 11, 1916; excellent roots; no galls.

[10] Removed September 11, 1916; root system fair; crown somewhat enlarged but gall practically disappeared.

[11] Removed September 1, 1915; severe borer injury; gall disappeared.

TABLE 2 (*continued*)

Variety	Location	1911 Oct. 3	1912 Oct. 12	1913 Sept. 6	1914 Sept. 14	1915 Sept. 14	1916 Sept. 11	1917 Sept. 25
Carman..............	C- 9	Dead[2]	.....	.....	.....	.....	.....	......
Carman..............	C-10	.....	f	g	f	g	g	g
Carman..............	C-11	Dead[2]	.....	.....	.....	.....	.....	......
Carman..............	C-12	Dead[2]	.....	.....	.....	.....	.....	......
Carman..............	C-13	.....	g	g	f	e	e	e
Carman..............	C-15	.....	g	g	f	f[12]	.....	......
Carman..............	C-16	.....	e	g	g	g[13]	.....	......
Carman..............	C-17	.....	e	e	e	e	e	e
Carman..............	C-18	.....	f	g	p[14]	p[15]	.....	......
Carman..............	D- 1	.....	e	e	e	e	e[16]	......
Carman..............	D- 2	.....	g	g	e	g	e	e
Carman..............	D- 3	Dead[2]	.....	.....	.....	.....	.....	......
Carman..............	D- 4	Dead[2]	.....	.....	.....	.....	.....	......
Carman..............	D- 5	.....	Dead[1]	.....	.....	.....	.....	......
Carman..............	D- 7	.....	e	e	e	e	e	e
Carman..............	D- 8	.....	f	p[14]	p	p	.....[18]	.....
Carman..............	D- 9	.....	g	g	g	e	e	e
Carman..............	D-10	.....	p[19]	g	....[20]	.....	.....	......
Carman..............	E- 1	.....	g	g	e	g	e	f
Carman..............	E- 2	.....	g	e	e	e	e	e
Carman..............	E- 3	.....	p[3]	g	e	e	e[21]	......
Carman..............	E- 4	Dead[2]	......	.....	.....	.....	.....	......
Carman..............	E- 5	.....	e	e	e	e	g[22]	......
Carman..............	E- 6	Dead[2]	.....	.....	.....	.....	.....	......
Carman..............	E- 7	.....	e	e	e	e	e	e
Carman..............	E- 8	.....	e	g	p	p	p[23]	......
Carman..............	E- 9	Dead[2]	.....	.....	.....	.....	.....	......
Carman..............	E-10	.....	g[3]	e	e	e	e	g
Carman..............	*E-11	.....	g	e	g	e	e	e
Carman..............	*E-12	.....	e	e	e	e	e	e
Carman..............	E-13	.....	e	e	e	e	e	e
Carman..............	E-14	Dead[2]	.....	.....	.....	.....	.....	......
Carman..............	E-15	Dead[2]	.....	.....	.....	.....	.....	......
Carman..............	E-16	.....	g	f	p[14]	f[24]	.....	......
Carman..............	E-17	.....	e	g	g	f[25]	.....	......
Carman..............	E-18	.....	g	g	g	e	e	e
Elberta...............	*C- 6	.....	g	g	g	g	e	g

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[12] Removed September 1, 1915; severe borer injury; gall disappeared; two galls on roots; hairy root above crown.

[13] Removed September 1, 1915; original gall disappeared; three galls (walnut) on one root.

[14] Borer injury.

[15] Removed September 1, 1915; original gall disappeared; one gall 1 centimeter in diameter on one root.

[16] Removed October 11, 1916; gall disappeared; large tree, large root system.

[17] Removed October 11, 1916; root crown in bad condition, evidently from gall but complicated by severe borer injury; three small galls on roots.

[18] Missing, no record; severe borer injury previously.

[19] Injured by plow.

[20] Dead July 1, 1914; no record.

[21] Removed October 11, 1916; borer injury; several small galls.

[22] Removed October 11, 1916; borer injury severe; galls disappeared.

[23] Roots fair; three small galls and indications of hairy root.

[24] Removed September 1, 1915; original gall disappeared; one gall 1 centimeter in diameter on one root.

[25] Removed September 1, 1915; borer injury; gall disappeared; indication of hairy root.

TABLE 2 (*concluded*)

Variety	Location	1911 Oct. 3	1912 Oct. 12	1913 Sept. 6	1914 Sept. 14	1915 Sept. 14	1916 Sept. 11	1917 Sept. 25
Elberta	C-14	.....	f[3]	g	g	g	g[26]	......
Elberta	D- 6	.....	f[3]	g	f	g	g[17]	......
Elberta	F- 1	.....	g	f	p[27]	.....	.....	......
Elberta	F- 2	.....	g	g	e	e	e	e
Elberta	F- 3	Dead[2]	.....	.....	.....	.....	.....	......
Elberta	F- 4	Dead[2]	.....	.....	.....	.....	.....	......
Elberta	F- 5	.....	f	f	f	f[28]	.....	......
Elberta	F- 6	.....	e[3]	g	e	e	e	e
Elberta	F- 7	Dead[2]	.....	.....	.....	.....	.....	......
Elberta	F- 8	.....	e	g	e	f[29]	.....	......
Elberta	F- 9	Dead[2]	.....	.....	.....	.....	.....	......
Elberta	F-10	.....	g	g	g	f	f	f
Elberta	G- 1	.....	f	f	e	g	g	e
Elberta	G- 2	Dead[2]	.....	.....	.....	.....	.....	......
Elberta	G- 3	Dead[2]	.....	.....	.....	.....	.....	......
Elberta	G- 4	.....	e	e	g	e	e	e
Elberta	G- 5	.....	e	e	g	g	e	e
Elberta	G- 6	.....	e	e	g	e	e	e
Elberta	G- 7	.....	g	e	e	e	g	e
Elberta	G- 8	.....	e	e	g	e	g	f
Elberta	G- 9	Dead[2]	.....	.....	.....	.....	.....	......
Elberta	G-10	.....	g[3]	g	f	g	f[30]	......
Elberta	*G-11	.....	g[3]	f	p[14]	p[31]	.....	......
Elberta	*G-12	.....	g[3]	g	g	f	p[32]	......
Elberta	G-13	.....	e	g	g	g	e	e
Elberta	G-14	.....	g[3]	g	p[14]	f	p[33]	......
Elberta	G-15	.....	g[3]	g	g	g	e	e
Elberta	G-16	.....	g	g	g	f	e	e
Elberta	G-17	.....	e	g	f	f	e	e
Elberta	G-18	.....	g	f	g	f	g[34]	......
Elberta	H- 8	Dead[2]	.....	.....	.....	.....	.....	......
Elberta	H- 9	Dead[2]	.....	.....	.....	.....	.....	......
Foster	H- 1	Dead[2]	.....	.....	.....	.....	.....	......
Foster	H- 2	Dead[2]	.....	.....	.....	.....	.....	......
Foster	H- 3	Dead[2]	.....	.....	.....	.....	.....	......
Foster	H- 4	Dead[2]	.....	.....	.....	.....	.....	......
Foster	H- 5	Dead[2]	.....	.....	.....	.....	.....	......
Foster	*H- 6	.....	e[3]	g	g	f	g[35]	......
Foster	*H- 7	.....	e	e	e	e	e	e

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[26] Removed October 11, 1916; no record.

[27] Removed July 1, 1914; poor location and practically dead.

[28] Removed September 1, 1915; frost injury; gall disappeared.

[29] Removed September 1, 1915; injured by frost and borers; old gall not apparent.

[30] Removed September 11, 1916; borer injury; roots good; large galls, 6 to 7 centimeters in diameter, on crown and roots.

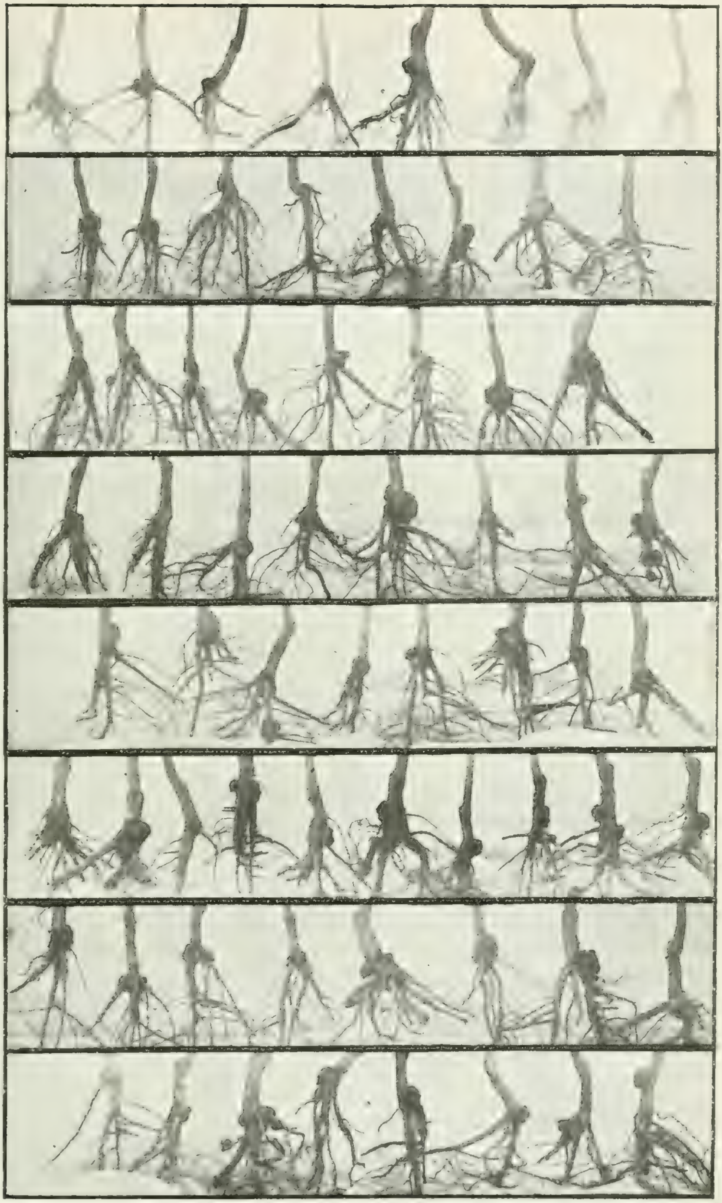
[31] Removed September 1, 1915; injured by borers and frost; practically dead.

[32] Removed September 11, 1916; severe borer injury; no galls.

[33] Removed September 11, 1916; borer injury severe; roots small; three small galls.

[34] Removed September 11, 1916; borer injury severe; one small gall.

[35] Removed September 11, 1916; recovering from severe borer injury; no galls.



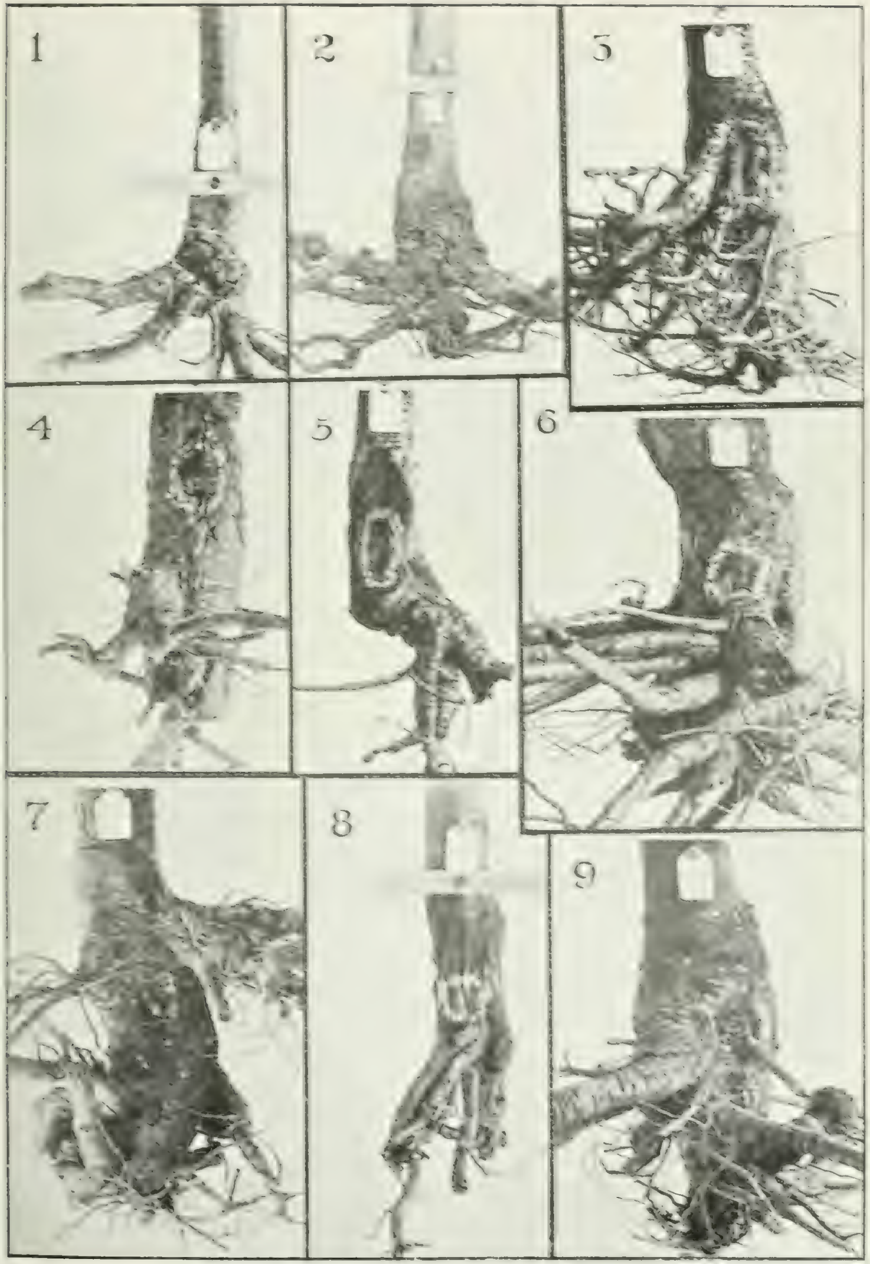
ROOTS OF THE PEACH TREES AFFECTED WITH CROWN-GALL WHICH WERE USED IN THE EXPERIMENT

The behavior of these trees in the field is shown in table 2. In each case read from left to right except in the row at the top, where tree 7 is tree 10 of the records and trees 8, 9, and 10 are 7, 8, and 9 of the records; and in the row at the bottom, where it is necessary to read from right to left. (Special acknowledgment is made to Mr. W. R. Fisher for his technical skill in obtaining suitable prints from most unsatisfactory negatives)

PLATE II. ROOTS OF PEACH TREES FROM THE CROWN-GALL EXPERIMENT

The condition of most of the roots at the time of setting is shown in Plate I, and a record of behavior previous to digging is given in table 2. In cases in which the original gall has disappeared, its location is outlined with chalk

1, Tree A-4. Removed September 1, 1915, because of winter injury. Gall apparently inactive
2, Tree A-11. Removed October 11, 1916, because of crowding. Crown considerably enlarged and several small galls, but tree apparently in good condition
3, Tree E-16. Removed September 1, 1915. A small gall at base of root issuing from near bottom of original gall
4, Tree A-18. Removed September 1, 1915, because of winter injury
5, Tree F-5. Removed September 1, 1915, because of frost injury. Gall disappeared, and no indication that it could have been a factor in decline of tree
6, Tree F-8. Removed September 1, 1915, because of injury by frost and borers
7, Tree G-11. Removed September 1, 1915, because of severe injury by frost and borers
8, Tree C-18. Removed September 1, 1915. Original gall disappeared
9, Tree C-16 Removed September 1, 1915. Original gall disappeared. Three new galls have appeared, two of which show clearly in the photograph



ROOTS OF PEACH TREES FROM THE CROWN-GALL EXPERIMENT

(See legend opposite)

GENERAL CONSIDERATIONS

Field experimentation is very unsatisfactory at best, because so many uncontrollable factors are involved which interfere with a correct interpretation of results. This condition has come to be rather generally understood, the recent analyses by Harris[10] of results from field plot tests, and by Anthony and Waring[11] for orchard tests, only serving to indicate how generally unreliable such data are. In addition to the usual vicissitudes encountered in plot testing, an additional set of variables is added when one attempts to compare plants in health and in disease. Means have been devised for overcoming heterogeneity in field plots, but not enough is known about plant responses under conditions of parasitism to make suitable corrections. This is particularly true in the case of the disease under consideration, because Smith has shown that the response to the stimulus of *Bacterium tumefaciens* depends a great deal on the particular tissue affected. This individual variation can be eliminated by increasing the number of plants under test. The number of plants involved in a wholly satisfactory experiment obviously should be considerably greater than was included in this experiment, and the arrangement should be different.

The general indication, however, is that under the conditions of this experiment, crown-gall is not a factor in the growth and development of either apple or peach trees.

The agitation among fruit growers in regard to crown-gall has practically disappeared, doubtless due to the inspection service which causes rejection of trees bearing galls. The writers, however, in 1914 received a consignment of 400 apple trees, of which 28 (7 per cent) bore definite lesions of crown-gall. All of these were planted on private land and their exact location was recorded. While it is likely that many growers treat galled trees just as the writers did, it is to be recalled that the amount of crown-gall on nursery stock has not been large, and the rejection of trees on account of crown-gall has not been a serious loss to nurserymen.

The rôle of crop rotation in reducing losses from crown-gall in the nursery is an interesting phase of the problem, on which, unfortunately, experimental data are too meager to warrant any conclusions.

---

[10] Harris, J. Arthur. Practical universality of field heterogeneity as a factor influencing plot yields. Journ. agr. res. 19:279–314. 1920.

[11] Anthony, R. D., and Waring, J. H. Methods of interpreting yield records in apple fertilization experiments. Pennsylvania Agr. Exp. Sta. Bul. 173:1–42. 1922.

BIOLOGICAL STUDIES OF BACTERIUM TUMEFACIENS

There are a number of biological problems connected with an investigation of crown-gall which are of considerable practical importance. One of the writers (S) was engaged in such work. He had undertaken an extensive series of tests designed to determine the longevity of the crown-gall organism in pure and in mixed culture, and under a variety of conditions. These were not completed when his connection with the institution was severed, and there was no opportunity to finish them before his untimely death intervened. The data accumulated are fragmentary, but their significance warrants publication at this time. They are given in the following paragraphs.

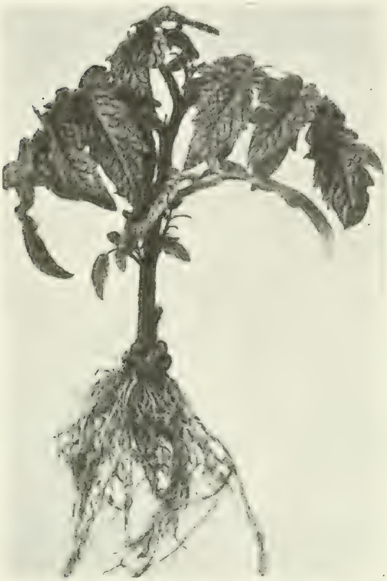


FIGURE 4. CROWN-GALL ON TOMATO PLANT

The cultures of *Bacterium tumefaciens* recovered in the studies on vitality were tested in this way in order to confirm identification

Persistence of B. tumefaciens in sterilized soil.— In order to determine the persistence of the crown-gall organism in sterilized soil, the following experiment was begun on November 10, 1914. Fifteen small pots were filled with loamy soil and sterilized. Five cubic centimeters of a bouillon culture of *B. tumefaciens*, four days old, was added to each pot. Twelve test tubes were partially filled, some with loam, others with quartz or clay, and to these one cubic centimeter of the culture was added. After four days all pots and tubes were tested to determine the presence in living condition of the organism (figure 4). All were then placed in a box out of doors. On January 2, 1915, the organism was recovered from clay, loam, and quartz. On April 14, 1915, a test of the loam soil in the test tubes showed the organism to be present in a living condition. Again on May 24, 1915, the organism was found to be alive in a pot of loam and in a tube of quartz. The next test was made on September 14, 1915. The organism was not recovered from either pots or tubes.

Persistence in unsterilized soil.—Experiments similar to the preceding were instituted with clay, loam, and quartz which had not been sterilized. As this was the most important series from the practical standpoint, it is unfortunate that data on the outcome cannot be found among Dr. Stewart's notes.

Moisture relation.—On February 24, 1914, tests were instituted to determine the vitality of *B. tumefaciens* in dry, moist, and saturated clay, loam, and quartz. Sterilized material in test tubes was used, and the tubes were constantly covered with a bell glass to prevent evaporation. On March 11, 1914, the organism was alive in all nine tubes. On April 10, 1914, the three tubes of dry material were tested and the organism was recovered. In a duplicate set of tubes which were not covered, the organism was dead on the latter date in the dry clay and the dry quartz. All the protected tubes were tested on November 18, 1914, and *B. tumefaciens* was found to be alive except in the tubes of dry clay and dry quartz. Sterilized wheat seeds were planted in some of the tubes of moist loam on April 17. The seeds grew and developed into plantlets. On May 29 a test was made of these tubes and *B. tumefaciens* was found to be alive.

Penetration of soil.—Tubes were filled with moistened quartz and with moist loam, and were sterilized. Five cubic centimeters of a culture of *B. tumefaciens* was poured into one end of each tube, and enough sterilized water was added so that water would drip from the other end. This was caught under aseptic conditions, and at the end of forty-eight hours was tested by plating. The organism was recovered from tubes 60 centimeters in length. No greater lengths were tested, as 60 centimeters represents the approximate penetration into the soil of roots of young trees. Similar tests with clay gave penetration in some instances and not in others. Apparently, if the clay became "puddled" from adding water too rapidly, the organism was filtered out.

While these tests are not extensive, they certainly indicate that, when free from competition, *B. tumefaciens* can live for several months in moist soil of various types, that it can withstand low temperatures and repeated change in temperature at or near the freezing point, and that it may move considerable distances with currents of soil water.

Memoir 67, ***Observations on the Life History of Taphrocerus gracilis (Say) (Beetle, Family Buprestidae)***, the sixth preceding number in this series of publications, was mailed on September 14, 1923.

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WIRE STEM OF CABBAGE

LEVI OTTO GRATZ

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WIRE STEM OF CABBAGE[1]

LEVI OTTO GRATZ

The cabbage crop ranked sixth in 1919 and 1920, and seventh in 1921, in the number of commercial acres produced among fifteen leading truck crops grown in the United States. During that time New York produced 23,287 acres of late cabbage per annum, with an average yield of 8.2 tons per acre.[2] In this State the crop is second only to potatoes, and is the most important of all the other vegetables grown.[3] Its total value, based on average prices from 1920 to 1923, inclusive,[4] approximates three million dollars yearly. From 1919 to 1921, inclusive, New York ranked ninth in yield per acre among twenty-one leading States for which figures are available.[2]

It is an established fact that the early market bids the highest prices. This is especially true for the early crop. On the Buffalo wholesale curb market, in the vicinity of which these investigations were first undertaken, it is not uncommon for the growers to sell their first cabbage about the middle or latter part of July for eight cents or more per head, and several weeks later to sell cabbage of higher quality for less than half this price. Later in the season an overstocked market occasionally forces the grower to return from market with part of his load unsold. It is therefore apparent that a short delay in the maturing of the early crop may mean a large loss to the grower. There are numerous factors which may cause such a delay, as, for example, adverse environmental conditions or disease of the seedlings.

THE DISEASE

NAMES, HISTORY, AND GEOGRAPHICAL RANGE

The general term *damping-off* may be applied to the early stage of this particular disease. By this term is meant the killing of the tissues of the seedling at the surface of the ground so that eventually the plant topples over. More specific names, as *black rot* (Duggar and Stewart, 1901),[5] *black shank disease*,[6] and *stem rot* (Fawcett, 1909), have been applied to the disease in the past. The last-named term is most frequently used in western New York. These names are used rather than the term *damping-off*, since diseased seedlings often do not topple over and may apparently recover. In fact, plants with slight lesions, though undoubtedly permanently injured, are frequently planted in the field. None of these terms appear to the writer to be sufficiently descriptive. *Damping-off* is too general and applies to the early stage only. *Black rot* is already in general use for the bacterial disease caused by *Pseudemonas campestre* (Pammel) E. F. Smith. *Black shank disease* only partially conveys the idea intended. *Stem rot* is inaccurate, as the stem may not exhibit a pronounced rot, especially in the later stage of the disease. In view of these facts the writer suggests the name *wire stem*. This is more desirable since most of the plants are not killed outright by the primary attack, but emerge with a characteristically tough, wiry stem (Plate I). This name accurately conveys the idea suggested by the symptoms.

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[1] Also presented to the Faculty of the Graduate School of Cornell University, June, 1923, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

AUTHOR'S ACKNOWLEDGMENT. The writer wishes to acknowledge his indebtedness to Professor L. M. Massey, under whose immediate direction this work was performed, and to the members of the departmental staff, for many helpful suggestions and criticisms.

[2] U. S. Dept. Agr., Yearbook **1921**:647–649. 1922.

[3] Fourteenth census of the United States, taken in the year 1920, vol. 5, p. 820–831. 1922.

[4] U. S. Dept. Agr., Weather, Crops, and Markets **4**:680. 1923.

[5] Dates in parenthesis refer to bibliography, page 59.

[6] U. S. Dept. Agr., Bur. Plant Ind., Plant disease bul. 1, p. 49. 1917.

The first definite reference to this disease was by Atkinson (1895). He describes in detail the damping-off of seedlings of such plants as radishes and lettuce, and states that " egg plants and cabbages as well as others are known to be affected." He attributes this to a " sterile fungus " now known as *Corticium vagum* B. & C. Duggar and Stewart (1901) report the disease on cabbage seedlings from Cairo, Illinois, in 1898, and also on cauliflower seedlings at Geneva, New York. They consider *Corticium vagum* the causal organism. Fawcett (1909) observed the damping-off of cabbage seedlings in Florida, caused by this fungus. Six years later Sherbakoff (1916) reported isolating the fungus from cabbage, cauliflower, and other hosts in Florida, and during the following two years he presented data concerning some phase of experimental work on damping-off (1917, a and b). During the past few years the disease has been reported from Washington, Utah, Missouri, Indiana, Ohio, Alabama, Louisiana, Virginia, and probably several other States.

ECONOMIC IMPORTANCE

It is difficult to obtain definite information concerning the actual losses caused by this disease. The occurrence and the severity of the disease where early cabbage is grown are not uniform, thereby making estimates of actual seedling losses impossible. A severe outbreak is especially disastrous to the grower of large acreages because of the large number of plants (approximately 10,000) required to plant an acre. A total or partial loss of the seedlings with the season far advanced results in a reduced acreage, as there is but slight possibility of obtaining enough seedlings from neighboring growers to replace those that are killed. To forestall such an emergency a much larger number of seedlings are grown than would otherwise be required. It is evident that this requires much additional time and greenhouse space.

In 1920 the writer endeavored to arrive at some more definite conclusions concerning the actual losses caused by this disease. Counting more than 1000 plants on each of three farms, immediately before transplanting into the field, it was found that 55 per cent of the seedlings were healthy, while 23 per cent were slightly diseased, and 22 per cent were severely diseased. This arbitrary classification of the diseased plants was made from the standpoint of the grower, who normally sets the slightly diseased plants and discards those designated as severely diseased. Some of the growers were of the opinion that slightly diseased plants which had apparently recovered could be transplanted into the field without any subsequent losses. To demonstrate that permanent injury had resulted to the seedlings, several hundred of each of the above classes of plants (healthy, slightly diseased, and severely diseased) were planted in adjacent rows on each of two farms. Careful records were kept of the number of heads and the pounds produced by each class. It was estimated that the actual losses on the two farms, considering the healthy plants as yielding normally, were 12 per cent in heads and 10 per cent in pounds for the slightly diseased plants, and 36 per cent in heads and 30 per cent in pounds for the severely diseased plants. If, then, of the 10,000 plants required to plant an acre, 23 per cent (2300 plants) were slightly diseased, the actual decrease in yield in heads (12 per cent) from this class only was 276 per acre. Accepting five cents a head as the average selling price, the loss per acre was approximately $14. This is a loss of which most growers are probably not aware. In addition to this loss and that of the discarded plants, there is also a loss caused by a delay in maturing of heads produced by the diseased plants. This loss is dependent on the fluctuations of the market, and may assume rather extensive proportions. This is especially true in a season with an early market. In 1920 there was an excessive amount of rainfall throughout the growing season, and this is probably the reason why there were only slight indications of delay in maturing. The following year an attempt was made to duplicate the above experiments, but the disease was not severe on any of the farms. On one, however, 32 per cent of the plants were found to be very slightly affected. Several hundred of these were planted, as before. Because of the only slightly diseased condition of the seedlings, the results of the harvest did not demonstrate any actual loss in yield, but a definite delay of a week or ten days in the ripening of the heads was very evident.

SYMPTOMS

Wire stem of cabbage is first evident as a typical damping-off. Usually the disease is to be observed when the seedlings are an inch or two in height. A water-soaked area completely encircling the tender stems is found just at the surface of the soil. In a few hours the stem loses its supporting power and the plant topples over. This occurs especially where the soil is heavily infected with the causal organism. Under these conditions germination of the seed may be prevented, or the plants may be killed outright, but usually most of them will recover partially. In light attacks there may be no toppling over. If the moisture content of the soil is high, the plants may not even wilt. In a very few days, the cortex will be destroyed partially or completely and the second stage of the disease, wire stem, will be evident. Now is to be found a crooked, dwarfed plant with a peculiar tough, woody, wire-like stem (Plate I). Small brownish lesions, similar to those caused by this fungus on potato stems, are seldom found, but usually the entire basal part of the stem is affected. In the earlier, or damping-off, stage, the mycelium of the fungus is easily demonstrated at the margin of the lesion. When the wire-stem stage has appeared, it is often impossible to do this especially in the later period of the disease.

ETIOLOGY

Nomenclature, and review of literature

The disease under consideration is caused by *Corticium vagum* B. & C. The Corticium stage of this fungus was first recorded by Rolfs (1903, 1904). Judged by its parasitic mode of life and morphology, it was considered sufficiently different from *Corticium vagum* B. & C. to constitute a new variety and was designated *Corticium vagum* var. *solani* Burt. Burt (1918) later identified the Corticium causing the potato disease as the common *Corticium vagum* B. & C., thus reducing the variety to synonymy. Duggar (1915, 1916) reviews extensively the early European literature. and concludes that the fungus causing damping-off in this country is the same as *Rhizoctonia solani* Kühn, which causes lesions on potato stems in Europe. He states that the fungus is distributed throughout the United States, Canada, and Europe, has been reported from Brazil, France, Ireland, Australia, Great Britain, New Zealand, and Japan, and probably has been observed in all its stages in the more humid regions of India. Peltier (1916) gives an extensive table, setting forth the distribution of the disease and its many scores of hosts in the United States and other countries. Matsumoto (1921) was concerned chiefly with specialization of *Corticium vagum*, both in his review of literature and in his researches. This phase is discussed later in this paper.

Morphology and physiology

The mycelium

The mycelium of the fungus causing wire stem of cabbage is, both within the host and in culture, the typical, septate, broad, hyaline, branching "sterile fungus" mycelium which Atkinson (1895), Rolfs (1902, 1904), and others have accurately described. The diameter of the hyphae as computed by measuring fifty strands of a five-days-old culture is approximately 8.5 microns. This measurement was compared with others similarly obtained from six strains[7] of the same age, isolated from potato tubers and grown on the same medium.[8] No difference in size was observed (table 1).

The strain used throughout this work was isolated from a cabbage seedling in 1920. In 1923 lettuce seedlings were inoculated with this strain, and the fungus was reisolated. The average diameter of the hyphae of the fungus more recently isolated was not different from that of the fungus grown on potato agar for over two years. Comparisons were made also of the diameters of hyphae of strains from cabbage, bamboo, and aster plants. The hyphae of the last two were consistently of smaller diameter (table 1).

TABLE 1. Measurements of Hyphae and Sclerotial Cells, in Microns, of Different Strains of Corticium vagum B. & C.

Strain	Culture		Average diameter of mycelium (microns)	Dimensions of sclerotial cells (in microns)		
				Extremes		Average size
	Age	Source		Width	Length	
Cabbage	3 years	Seedling	8.5	16–28	22–38	19×30
Cabbage	1 month	Seedling	8.8	16–26	22–39	20×29
Potato 27	1 month	Tuber	8.5	16–30	22–41	23×31
Potato 49	1 month	Tuber	8.6	16–30	22–36	20×29
Potato 13	1 month	Tuber	8.8	15–27	24–44	22×32
Potato 70	1 month	Tuber	9.2	13–24	22–41	20×33
Potato 55	1 month	Tuber	9.3	16–30	23–43	23×32
Potato 51	1 month	Tuber	9.5	16–30	22.41	22×29
Aster	4 months	Seedling	6.2	10–20	15–24	14×19
Bamboo	1 year	Bud	7.3	8–13	14–24	11×19

The sclerotial cells

The barrel-shaped sclerotial cells so characteristic of *Corticium vagum* were present in abundance in seven-days-old cultures on potato agar (pH adjusted to 5.6). Comparisons of the average length and width of fifty of these cells were made of the strains mentioned above. Again the only differences in size were found in the consistently smaller cells of the strains from the bamboo and aster plants (table 1). The indications were also that the cells of these two strains were different in shape, but the extreme variation in any one of the strains makes it impossible to state with certainty that this was uniformly the case. Daily microscopical examination of the cultures (potato agar) up to the seventh day revealed the fact that the sclerotial-cell formation was considerably slower in the strain from aster, and still slower in the strains from bamboo and cabbage, than in any of the strains from potatoes. In the strains from potatoes the rate of this formation was about uniform.

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[7] The term *strains* is used to designate isolations from different sources, as different tubers, different hosts, and so forth.

[8] The following formula was used in the preparation of the medium: pared potatoes, 200 grams; dextrose, 10 grams; agar, 20 grams; tap water, 1000 cubic centimeters.

The fruiting stage

Two forms of hyphal growth similar to those described by Rolfs (1904) were frequently associated with wire stem. The light, hyaline form was usually found within the cortical tissue of the plant attacked. Frequently the dark form was found growing about the base of the seedling. With the potato strains, on potatoes, this was especially noticeable. In the inoculation experiments on potatoes, a large proportion of the stems were covered with this dark, weblike mycelium. An unsuccessful attempt was made to bring this mycelium into fruiting by placing the plants under bell glasses. With the cabbage strain, on crucifers, this mycelium was also very prevalent. The typical, white, granular, fruiting layer appeared frequently on the small stems of the cabbage, turnip, and radish seedlings, but it was only in one case, on turnips, that the basidia, with the sterigmata and the spores, were actually demonstrated. Several basidia, but only two spores, were observed. This limited number of fruiting structures is not regarded as sufficient to identify the organism, but from the measurements and general appearance they were considered identical with those described by Rolfs for the perfect stage of *Corticium vagum.* At no time in the numerous inoculations made was this "fruiting" mycelium from the potato strains observed on the cabbage seedlings, or that of the cabbage strain on the potato stems.

The temperature range for growth

Accurately controlled temperature chambers were available for only the higher temperatures. A fair idea concerning the minimum temperature for growth was obtained by placing petri-dish cultures in the bottom of a refrigerator where the temperature limits were about 9° to 11° C. Other cultures were placed in about the center of the refrigerator, where the temperature limits were approximately three degrees higher, and still others at the top, where the fluctuation was somewhere between 14° and 16° C. Another set of petri-dish cultures was held at room temperature, and others in controlled temperature chambers. The minimum and maximum temperatures for growth were found to be approximately 9° and 31° C. The optimum is not sharply defined, but is somewhere between

22° and 26° C. (table 2). The figures given in the table represent the averages of three trials.

TABLE 2. COMPARISON OF GROWTH OF HYPHAE OF THE CABBAGE STRAIN OF CORTICIUM VAGUM AT DIFFERENT TEMPERATURES, THREE DAYS AFTER BEING TRANSFERRED

Temperature (centigrade)	9°–11°	11°–14°	14°–16°	17°–23°	21.5°	22.5°	25.5°	27.0°	28.0°	29.0°	32.0°
Growth (centimeters)	0.5	1.3	3.0	4.0	6.3	7.0	8.0	6.0	3.5	1.2	0.5

The pH range for growth

The relation of the growth of the fungus to the hydrogen-ion concentration in culture was determined by making transfers from a young culture to petri dishes containing a freshly made medium adjusted to the desired acidity or alkalinity, and measuring the diameter of the area covered after five days of growth. Exactly 200 cubic centimeters of freshly prepared potato agar was placed in flasks. The proper number of drops of M/5 HCl or M/5 NaOH to produce the desired pH in each flask, as determined roughly by a preliminary trial, were placed in test tubes plugged with cotton. One tube was prepared for each flask. Where only a few drops were to be added to the medium, about 5 cubic centimeters of distilled water was placed in the tube to facilitate the delivery of the reagent from the tube to the flask. Several tubes containing 10 drops of either the acid or the alkali were also prepared as reserve tubes. In case the original amounts of reagent added did not produce the desired hydrogen-ion concentration, these known amounts were added to the partially adjusted media while it was still warm, without unnecessary delay or reheating. Both flasks and tubes were autoclaved for fifteen minutes at 15 pounds pressure. Without being permitted to solidify, the medium of each flask was adjusted by pouring into it the reagent from the corresponding tube and the pH was determined. In other trials, in which it was impossible to complete the adjustment for lack of time, the medium was not adjusted at the time of sterilization, but was allowed to cool and was reheated when the entire operation could be completed. The purpose of this was to eliminate complications which would result from intervening cooling and heating. The pH determination was made colorimetrically, with buffer mixtures,[9] and with the aid of a comparator block.[10] Several samples of 5 cubic centimeters each were poured (not pipetted) into test tubes, allowed to cool, and compared with the standard indicators previously prepared. The medium of each flask was poured into petri dishes before the adjustment of the next flask was begun. This method is particularly satisfactory because the dangers of contamination are reduced to a minimum, and because it permits large numbers of replications on media of exactly the same pH value.

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[9] Clark, W. Mansfield. The determination of hydrogen ions, p. 1–317. 1920.

[10] Gillespie, Louis. Colorimetric determination of titration curves without buffer mixtures. Amer. Chem. Soc. Journ. 42:742–748. 1920. *Also*, Colorimetric determination of hydrogen-ion concentration without buffer mixtures, with especial reference to soils. Soil sci. 9:115–136. 1920.

In a preliminary experiment performed as described, with the exception that lactic instead of hydrochloric acid was used for acidification, it was demonstrated that the minimum and maximum pH values for four different strains of the fungus were beyond 2.4 and 8.5, respectively. The optimum was not sharply defined. Considerable variation was in evidence, both within each strain and between the different strains.

In a subsequent experiment the same strains were compared, and the above-mentioned aster strain also was included. Considerable variation, due to some unexplained factors, again resulted. This experiment, however, clearly demonstrated that the maximum and the minimum were a little above and a little below pH 10.0 and 2.4, respectively. Again the optimum was not sharply defined.

In a third trial, both the old 1920 culture and the freshly isolated 1923 culture of the cabbage strain were compared with the aster, the bamboo, and the six potato strains. In this experiment the acid limit was reached for all the strains, but for neither strain was the alkaline limit definitely demonstrated (table 3). The rate of growth for the cabbage strain at the different pH values for five days is illustrated in Plate II. Considerable variation was again exhibited by the same strains at the different values. In general, the growth rate increased with an increase in pH up to a definite value, but rather frequently a plate of higher pH would show less growth than the one immediately below (table 3). A more sharply defined difference, however, was that of the tolerance of the various strains to acid media. The strain from bamboo was definitely more tolerant than any of the others, as it produced a mycelial growth one centimeter in diameter in four days at pH 2.0. The strains from potato tubers produced less growth, consistently, than either the cabbage, the bamboo, or the aster strain at this hydrogen-ion concentration (table 3). The color of the mycelium at these high acidities was white, so that it is not evident in the photographs of the petri dishes. By comparing table 4 and Plate III, it will be observed that at pH 2.4, the mycelium of both the cabbage and the aster strain completely covered the petri dish in twelve days. The potato strain (P 27) had covered an area of small diameter, almost completely covered with sclerotia, while the bamboo strain was a fluffy mass of white mycelium with minute sclerotia scattered over the surface of the petri dish (Plate III). At a pH of 2.7, similar results were again obtained. Here one of the potato strains (P 70) had covered an area of only 2.6 centimeters in diameter, while practically all of the other strains had covered the petri dishes (Plate IV).

TABLE 3. RATE OF MYCELIAL GROWTH OF CABBAGE, BAMBOO, ASTER, AND SIX POTATO STRAINS, OF CORTICIUM VAGUM ON MEDIA ADJUSTED TO DIFFERENT pH VALUES

Drops of reagent per 200 cubic centimeters of medium	pH	Growth, in centimeters, after four days									
		Strains*									
M/5 HCl		C 1920	C 1923	P 27	P 49	P 13	P 70	P 55	P 51	B	A
900	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
630	2.4	1.3	1.4	0.3	0.5	0.3	0.1	0.2	0.0	2.0	2.4
500	2.7	2.0	4.0	1.3	2.2	0.8	2.6	2.7	0.3	4.0	4.3
380	3.0	2.6	4.4	1.4	3.5	0.9	3.0	2.0	0.3	4.0	4.0
240	3.6	4.2	5.2	4.4	4.0	0.4	4.0	5.0	2.0	4.3	4.2
110	4.4	6.0	6.6	6.3	4.2	0.5	6.0	6.9	6.0	6.9	6.1
44	4.7	6.6	7.6	6.5	6.0	7.4	6.6	6.9	4.4	8.0	7.8
33	5.2	6.9	7.8	6.5	7.0	6.6	5.0	7.0	4.5	7.4	8.5
0	5.6	6.0	8.5	6.6	7.0	7.8	5.0	8.0	5.0	8.4	8.0
10	5.8	7.0	8.5	...	7.0	5.8	6.0	7.4	4.5	7.8	8.5
20	6.2	7.8	8.7	7.4	7.0	6.5	7.0	6.0	5.0	8.0	7.5
30	6.8	7.2	8.6	7.9	7.0	5.6	6.2	4.0	4.0	8.2	8.5
45	7.2	6.2	8.5	7.7	6.5	5.8	8.0	5.6	3.8	8.0	8.5
95	8.2	6.0	7.8	7.5	6.4	4.7	3.6	...	5.6	7.5	8.4
120	8.8	4.2	8.0	4.4	6.3	5.0	6.5	6.4	4.6	6.4	5.4
150	9.2	4.1	6.5	3.0	6.2	5.6	4.0	5.6	4.0	4.6	3.4
190	9.8	3.5	6.2	6.0	6.0	6.0	4.0	6.6	3.3	4.0	2.9
250	10.0	3.6	5.3	5.3	6.0	5.2	6.0	6.0	3.7	3.0	1.6
300	10.4	2.2	4.5	5.2	6.0	5.0	6.0	7.4	3.0	2.9	0.3
M/5 NaOH											

* C 1920, original strain from cabbage seedling.
C 1923, original strain reisolated from lettuce seedling.
P 27, strain isolated from potato tuber, from Elba, New York.
P 49, strain isolated from potato tuber, source unknown.
P 13, strain isolated from potato tuber, from Maine.
P 70, strain isolated from potato tuber, from college farms.
P 55, strain isolated from potato tuber, from college farms.
P 51, strain isolated from potato tuber, from college farms.
B, strain causing a bamboo bud rot in Bermuda.
A, strain isolated from aster seedling in college greenhouse.

The production of sclerotia in culture

The strains of the fungus at a pH of 2.7 exhibited a variation in the rate of formation of sclerotia as indicated in tables 4 and 5 and in Plate IV. There had been no indications previously, when they were grown in test tubes, that the several strains would produce sclerotia at a different rate; but when this comparative study was made, entirely different responses were noted in all three trials. The data in table 5 indicate the rate of sclerotia formation at the various pH values for the different strains. This is further illustrated in Plate V. The potato strain, typical for all six tested, is shown to be the most rapid, followed by the aster strain, while but very few minute sclerotia had been produced by the cabbage and bamboo strains. This was consistent for each of the seventeen petri dishes per strain, where sclerotia were produced. Another sharply defined difference was noticed between the sclerotia of the different potato strains themselves, namely, in the habit of their formation in the petri dishes (Plate VI). Those produced by strain P 13 were uniformly scattered

TABLE 4. RATE OF GROWTH AND FORMATION OF SCLEROTIA OF STRAINS OF CORTICIUM VAGUM AFTER TWELVE DAYS, ON VARIOUS ACID MEDIA *

Drops of reagent per 200 cubic centimeters of medium	pH	Strains †									
M/5 HCl		C 1920	C 1923	P 27	P 49	P 13	P 70	P 55	P 51	B	A
900.......	2.0	0	0	0	0	0	0	0	0	1.7 w	0
630.......	2.4	7.4 w	7.6 w	1.6 w	0.4 w	2.5 w	0.8 w	2.4 w	0.2 w	C w	8.5 w
500.......	2.7	C w	C w	C s w	C w	C w	2.6 s w	C s w	7.0 s w	C w	C w
380.......	3.0	C w	C w	C s w	C w	C s w	3.0 s w	C s w	8.5 s w	C b	C w
240.......	3.6	C w	C w	C s b	C s b	C s B	C s b	C s b	C s b	C b	C w
110.......	4.5	C b	C b	C s B	C s B	C s B	C s b	C s B	C s b	C b	C s b
44.......	4.7	C b	C b	C s B	C s B	C s B	C s B	C s B	C s B	C B	C s b
33.......	5.2	C b	C b	C s B	C s B	C s B	C s B	C s B	C s B	C B	C s b

* b, mycelium brownish in color; B, mycelium a deep brown; w, mycelium white; s, sclerotia present; C, entire plate covered with mycelium; numerals denote actual diameter, in centimeters, of the area covered with mycelium.

† Sources of strains as in table 3.

over the surface and were cinnamon brown in color. Strain P 70 produced large, uneven, irregular, ridge-like, grayish brown masses extending outward from the center of the dish. Strain P 27 formed blackish brown, closely compacted masses in the center of the petri dish.

TABLE 5. RATE OF FORMATION OF SCLEROTIA OF DIFFERENT STRAINS OF CORTICIUM VAGUM ON MEDIA ADJUSTED TO DIFFERENT pH VALUES

Drops of reagent per 200 cubic centimeters of medium	pH	Number of days after transfer of the culture									
M/5 HCl		C 1920*†	C 1923*	P 27	P 49	P 13	P 70	P 55	P 51	B *	A
900	2.0	0	0	0	0	0	0	0	0	0	0
630	2.4	0	0	12	0	0	10	0	0	0	0
500	2.7	0	0	10	12	9	6	7	10	0	0
380	3.0	0	0	10	12	9	6	7	9	0	0
240	3.6	0	0	10	12	6	5	6	8	0	12
110	4.4	0	0	8	9	5	5	5	7	0	11
44	4.7	0	0	5	8	5	5	5	7	0	11
33	5.2	0	0	5	8	5	5	5	7	0	11
0	5.6	0	0	5	8	5	5	5	7	0	11
10	5.8	0	0	0	8	5	5	5	7	0	11
20	6.2	0	0	5	8	5	5	5	7	0	11
30	6.8	0	0	5	8	5	5	6	7	0	11
45	7.2	0	0	5	8	5	5	6	7	0	11
95	8.2	0	0	5	8	5	5	6	7	0	12
120	8.8	0	0	6	8	5	5	6	7	0	12
150	9.2	0	0	6	8	5	6	6	7	0	12
190	9.8	0	0	6	10	5	6	6	7	0	12
250	10.0	0	0	6	10	6	6	6	7	0	12
300	10.4	0	0	6	10	6	6	6	7	0	12
M/5 NaOH											

* Very few minute sclerotia were formed in two or three plates in the twelve days during which the observations were made.

† Sources of strains as in table 3.

The foregoing results are in accordance with the researches of Matsumoto (1921) and others in that they point to constant differences. Since minor variations in environmental conditions apparently cause such large variations in growth, it would seem futile to attempt to compare these strains with those of another investigator.

In addition to observing the growth rate of the mycelium in the temperature experiments (table 2), the rate of sclerotia formation was also noted. Twenty days after the cultures had been transferred, the petri dishes at the intermediate temperatures were practically covered with sclerotia. Only a few had appeared at the second and third lowest temperatures, and none at the lowest and the three highest temperatures.

Pathogenicity

Historical aspects

According to Duggar (1915), Eidam (1888) was probably the first to mention the ability of *Corticium vagum* to cause a seedling disease of small plants. The host mentioned was the beet (*Beta vulgaris* L.). No complete account was given to prove that this fungus was the causal organism. Atkinson (1895), by his careful description of the general characteristics of the mycelium, and by his observations on the "sclerotoid bodies" produced by his "sterile fungus," leaves no question as to the identity of the organism. He, however, did no culture work to demonstrate its pathogenicity. Duggar (1899) attributes the damping-off of the sugar beet in New York to this fungus. Fawcett (1909), basing his conclusions on the identification of the fungus by Orton, who declares it the same organism as that on potatoes, attributes the damping-off disease of cabbage to *Corticium vagum* because of its constant association with the host. Sherbakoff (1916) is apparently the only worker who has made any inoculations of cabbage or other cruciferous seedlings to demonstrate that *Corticium vagum* is capable of producing the disease in question.

Experimental data

The writer first observed wire stem in April, 1920, on plants which were being transplanted into the field. Very few of these plants had been killed by the fungus, but a large amount of wire stem was found. In order to determine the source of the inoculum for infection under natural conditions, seeds previously disinfected with a 1–1000 mercuric-chloride solution for twelve minutes were planted in flats in (a) virgin soil from woods, (b) untreated soil from the greenhouse in which diseased plants had been grown, (c) soil from the greenhouse in which diseased plants had been grown and treated with either steam or formaldehyde. Several hundred plants were grown in each of these different soils. All of the plants in the virgin soil remained healthy, while all of those in the untreated greenhouse soil showed wire stem. Only a few plants in the treated greenhouse soil were affected. These results indicated that the soil carried the inoculum. Germination was greatly reduced in the infected soil, and an examination of the seed showed that many of the sprouts had emerged a millimeter or so and were then killed. Damping-off was noticed first. Within approximately ten days the plants that had not been killed outright developed wire stem. Later experiments confirmed these indications that the inoculum was harbored in the soil.

Finely chopped stems of diseased seedlings were next thoroughly mixed into virgin soil, and untreated seed was sown. Virgin soil not containing the diseased stems, and soil in which diseased plants had been growing, were used for comparison. The seed in the first flat germinated poorly. Most of the plants were killed outright, and the remainder developed wire stem. The seed in the fungus-infected greenhouse soil gave normal germination and considerable damping-off, followed by the wire-stem stage. The seed in the clean soil produced no diseased plants. This experiment indicates that parts of diseased plants may harbor the pathogene, that the fungus is not carried in the seed, and that the amount of inoculum in the soil is a factor in determining whether the plants will be killed outright or will develop wire stem only.

Diseased stems were next examined, and the typical, broad, hyaline mycelium of *Corticium vagum* was demonstrated in the outer tissues of the plant. Hundreds of tissue plantings from diseased stems were made on potato agar, and the fungus was consistently isolated throughout the summer.

In the winter of 1921, soil-infection experiments with pure cultures of the fungus were conducted, and the results obtained were conclusive in establishing the pathogenicity of the fungus. In addition, these experiments developed the most satisfactory method of inoculation. This method consisted in growing the fungus on sterilized wheat in wide-mouthed bottles, adding a kernel of this substrate to the base of the seedling, and covering slightly with soil. This procedure was followed exclusively in all of the later experiments unless otherwise stated. In the early experiments the inoculum was added to the soil directly, but this method was not satisfactory for wire-stem investigations as the number of plants pushing through the surface of the soil was greatly decreased.

In these early experiments the inoculum was added to thirty-six 4-inch pots of sterilized soil as follows: growing on potato agar, six pots; growing on wheat, fifteen pots; growing on soil-cornmeal mixture, fifteen pots. Six pots were maintained as controls Twelve treated seeds per pot were sown immediately after the inoculum was added to the soil. In the infected soil, germination and growth were very poor. In the controls, all of the seed germinated and the plants remained healthy. The check plants were inoculated later by adding the inoculum to the base of the stems, and all of them developed the disease. Isolations were made, and *Corticium vagum* and a species of Fusarium were obtained. In later inoculations only the former fungus produced the disease. Many such inoculations and reisolations in the past two years have removed all possibilities of doubt concerning the pathogenicity of this particular strain.

Inoculation experiments

Susceptibility of different varieties of Brassica oleracea L.

"All Seasons" was the variety of cabbage used in all of the preceding as well as the following experiments. Six early, nine late, four red, and three savoy varieties also were tested, and in addition five varieties of kale, four varieties of cauliflower, and one of brussels sprouts. None showed any degree of resistance. When the few plants that did not exhibit wire stem at the first count were reinoculated by placing some infected soil directly about the stems, damping-off occurred in every instance within twenty-four to forty-eight hours. After the observations were made, the checks were likewise inoculated, with similar results. Thus a total of about 250 seedlings of each variety were tested.

Susceptibility of other species of plants

The strain from cabbage was found to be pathogenic on eleven of the thirteen hosts inoculated (table 6). These hosts represent eleven species, eleven genera, and six families. No infection resulted when onion seedlings or potato stems were inoculated.

TABLE 6. Results of Inoculation of Different Hosts with the Strain of Corticium vagum from Cabbage

Family	Genus	Species	Common name	Number of pots	Number of plants	Per cent of disease*	Severity*
Cruciferae	Brassica	rapa L.	Turnip	5	100	80 d	......
Cruciferae	Raphanus	sativus L.	Radish	5	150	90 d	......
Chenopodiaceae	Beta	vulgaris L.	Beet	5	300	86 d	......
Leguminosae	Trifolium	pratense L.	Red clover	5	200	10 l	+
Leguminosae	Medicago	sativa L.	Alfalfa	5	300	17 l	+
Leguminosae	Vicia	sativa L.	Vetch	5	150	8 l	+
Leguminosae	Phaseolus	vulgaris L.	Bean	5	100	20 l	+
Liliaceae	Allium	cepa L.	Onion	5	50	0	......
Solanaceae	Solanum	melongena L.	Eggplant	5	100	80 l	++
Solanaceae	Solanum	tuberosum L.	Potato	65	65	0	......
Malvaceae	Gossypium	herbaceum L.	Cotton	5	50	20 l	++
Compositae	Arctium	minus Bernh.	Burdock	1	40	50 d	......
Compositae	Lactuca	sativa L.	Lettuce	2	120	80 d	......

* d, damping-off; l, stem lesions; +, very slight; ++, definite.

Susceptibility of plants of different ages

It is generally held that tender, succulent seedlings are very susceptible to damping-off, and that as the plants grow older this susceptibility gradually decreases. It is supposed that as the tissues become harder and older there is reached a stage of maturity after which the stems are practically immune. In order to obtain information on this point, seed was sown in sterilized soil at intervals of two weeks, and the plants were inoculated when the oldest had been growing for twenty weeks. No lesions were obtained on plants of that age, but very definite lesions were produced on those eighteen weeks old and younger (Plate VII). It is evident that the susceptibility gradually decreases with the age of the plant. Many of the lesions on plants over twelve weeks old were not typical for wire stem but were chiefly confined to a part of the stem only. A growing period of twenty weeks in the field is practically long enough to grow the early varieties to maturity. The variety used here, the late "All Seasons," requires a longer time for maturing.

Biologic specialization

Review of literature

It is a matter of common knowledge that *Corticium vagum* is parasitic on a wide range of host plants. The reader is referred to articles by Duggar (1915) and Peltier (1916) for comprehensive reviews of the literature on this phase of the subject.

Since the fungus is so widely distributed and occurs on such a great number of hosts, the question of strains may well be considered. Occasional reports of the discovery of definite strains emphasize the possibility that important differences may exist within the species. Duggar and Stewart (1901) early recognized the possibility of the existence of species or strains of the fungus, and conducted cross-inoculation work. They discontinued their experiments, however, before any definite conclusions were reached. Shaw (1912) isolated *Corticium vagum* from peanuts, cowpeas, jute, and cotton, and concluded from cross-inoculations that the organism from jute is restricted to that particular host. Duggar (1915), after a rather extensive study of the diseases caused by *Corticium vagum*, came to the conclusion that, while many of the strains from different hosts exhibit minor modifications, only that from rhubarb is of specific importance. He mentions the occurrence of strains, the evidence of which persisted for some time in the general appearance of the cultures. He also gives a brief statement concerning the variation in size of sclerotia, from the minute, scarcely visible ones, to those a centimeter or two in diameter.

In their publications in 1916, Duggar, Peltier, and Sherbakoff discussed the possibility of specialization. Duggar concluded that the common seedbed fungus in Germany and France is identical with the damping-off fungus which has frequently been observed since the investigations of Atkinson, and, further, that the damping-off fungus of this country is *Rhizoctonia solani* Kühn (*Corticium vagum* B. & C.). Peltier conducted extensive researches which included symptom studies; inoculation experiments involving comparisons of forty-five strains of the fungus on approximately 3000 cuttings, 2000 plants, and 7000 seedlings of various sorts; observations on artificial media; measurements of mycelial cells;

and soil surveys. He concluded from these experiments that all the strains studied by him were the same. These strains were obtained from a wide range of hosts from different parts of the country. Peltier observed no marked specialization in any of them. He did note, however, that the virulence of the fungus was extremely variable, as was also the degree of resistance of the various hosts. Growing the strains on artificial media, he observed much variation, those from the same host often producing different growth on the same substratum. However, the variations in cultural characteristics exhibited by isolations from different hosts were no greater than those manifested by two strains from the same host or by the same strain at different ages. Again, he could find no standard for distinguishing the different strains by means of mycelial cell measurements, because of the extreme variation in size even in the different isolations from the same host. Sherbakoff isolated *Corticium vagum* from more than fifteen hosts. He used eight strains, isolated from lettuce, castor bean, amaranthus, watermelon, cowpea, the pods of the garden bean and the velvet bean, and tomato fruit, and one culture received from Duggar, to inoculate seedlings of lettuce, celery, eggplant, pepper, and tomato. He found some strains pathogenic on cabbage, cauliflower, cucumber, garden bean, and other hosts, but it is not clear whether the fungus from potato was included in these. He concludes that the strains are morphologically alike, but makes no mention of any specialization in pathogenicity.

Rosenbaum and Shapovalov (1917), however, reported the isolation of a strain from potato which can be distinguished from the common form by the more pronounced lesions made when the strain is inoculated on injured stems or tubers; by the reaction, growth, and character of sclerotia on artificial media; and morphologically, by measurement of the diameter of germ tubes when the short, or "barrel-shaped," cells, enveloping the sclerotia, are placed in drops of water to germinate.

Edson and Shapovalov (1918) reported extreme variation in pathogenicity of the *Corticium vagum* strains used, from those absolutely unable to attack any underground parts to those which produced large and deep cankers. Also, not only were the size and the depth of the lesions consistently unlike, but their color and shape were consistently rather peculiar to certain individual strains. These investigators found that the strains in no way had become attenuated by reason of growth on artificial media for long periods. One in particular was very aggressive, being strongly parasitic on tomatoes and sugar beets, both as a damping-off fungus and in producing a root rot of adult plants.

Finally, the work of Matsumoto (1921), which is the most comprehensive and the most recent, must be considered. Fifteen isolations were obtained from different localities, from potato, lettuce, eggplant, *Habenaria* sp., and navy bean, and one from an unknown source. These were studied comparatively. From a macroscopic and microscopic examination some of them could apparently be identified conclusively, so that only six different types remained for physiological studies. All strains could hydrolyze starch and invert cane sugar. All showed increased growth at a pH lower than 7.0, and all grew abundantly at 3.8. Four of the six strains exhibited different minimum, optimum, and maximum temperatures for growth. Their diastatic activity was unlike, and there was a striking difference in the conversion of cane sugar. There were indications of differences in the cellulase activity of the different strains. A difference in the growth on casein, on peptone, and on asparagin was noted. Only two strains could utilize potassium nitrite. All strains exhibited different acid ranges. Fusion of hyphae was observed between some strains only, and a striking difference in virulence was observed. It was noted that greatest virulence was always manifest when inoculation was made on plants belonging to the same host as that from which the culture originated. Matsumoto concluded that there is a definite specialization among the strains tried, but that all are forms of *Rhizoctonia solani* Kühn except one, which may have exhibited sufficient differences to be considered of specific rank. This had been isolated from potato.

Reviewing, then, the whole problem in the light of these investigations, it is evident that the questions of biologic specialization needs further study. It might be of value to observe here that of the investigators who actually performed cross-inoculation experiments (Shaw, Sherbakoff, Peltier, Edson and Shapovalov, and Matsumoto), none, according to the evidence at hand, have inoculated cabbage seedlings with potato strains, or have used the cabbage strain to inoculate potato plants. Sherbakoff (1917a), working with but one strain from potato, may have conducted such cross-inoculations, as he states that some strains were tried on cabbage, cauliflower, cucumber, garden beans, and other plants, and proved able to produce damping-off.

Experimental data

Early in his experimental work, the writer found indications of the existence of specialization in the strains which he was using. In the spring of 1921, twelve cabbage seeds were sown in each of twenty-seven 4-inch pots of sterilized soil. When the seedlings were two inches high, they were inoculated by placing a bit of potato agar containing mycelium at the base of the stem and covering lightly with soil. The plants in six of the pots were inoculated with a strain from cabbage isolated the previous summer; those in six others, with another culture isolated at the same time; those in a third group of six, with a strain from potatoes from Canada; and those in a similar group, with a strain from potatoes from Maine. The remaining pots were kept as controls. Both the cabbage strains produced 100 per cent damping-off and typical wire stem. Neither of the strains from potatoes produced the disease. The checks likewise remained healthy. After ten days the inoculum was placed about the stems a second time, but again the plants remained healthy. Finally the cabbage strain was placed about the base of some of the seedlings, and all of these damped-off while the checks continued healthy.

The following June a similar experiment was performed in which twenty-two different strains of the fungus were tested. Twelve cabbage seeds were placed in each pot, and two pots were used for each strain. The strains were obtained as follows: thirteen isolated from as many different lots of potatoes obtained from New York, Minnesota, Maine, Canada, and some unknown sources; two from cabbage seedlings from western New York; one each from peas, peppers, eggplants, tulips, and grass; and two from unknown sources. Inoculations were made when the plants were two inches high. The cabbage, pea, pepper, and eggplant strains produced typical wire-stem lesions. All the others, including the checks, showed slight lesions, but these appeared to be a shriveling at the base rather than injury from fungus attack. When this experiment was performed, the temperature was abnormally high, and the possibility is that the high temperature, inadequate shading, and injudicious watering caused these peculiar atypical lesions. In this experiment the same Maine and Canada strains from potato which gave negative results in the previous experiment again produced no infection. The argument might be advanced that some of the strains were attenuated. While the exact date of isolation for some of them could not be determined, it is known positively that the cabbage, the eggplant, and some of the potato strains were of the same age.

In December, 1922, this experiment was repeated. As in the previous experiments, the exact length of time that the strains had been grown in culture was not determined, but most of them were of practically the same age. The cabbage strain, and some of the others also, were the same as those used in the preceding experiment. Five pots, each containing more than fifty plants, were used for each strain. The seed was sown in steam-sterilized soil, and the seedlings were inoculated when they were about two inches high. Here again the cabbage, pepper, pea, and eggplant strains produced typical wire-stem lesions, as did also the tomato, the salvia, and another strain of unknown origin (table 7). All of the plants inoculated with the potato and other strains, as well as the checks, remained healthy.

An experiment was then conducted to ascertain whether or not the strains from potato were pathogenic on potato. The soil of each strain from potato of the preceding experiment was mixed with an equal volume of sterilized soil. A tuber, or half of a tuber, of the Bliss Triumph variety, previously treated in a 1–1000 solution of corrosive sublimate for one and one-half hours, was placed in the bottom of each of ten pots, for each strain of the fungus. An additional amount of inoculum on wheat was

TABLE 7. RESULTS OF INOCULATIONS OF CABBAGE SEEDLINGS WITH DIFFERENT STRAINS OF CORTICIUM VAGUM

Strain	Source	Per cent of plants diseased
Pea	Wilson, New York	68
Lettuce	Williamson, New York	0
Potato	Unknown	0
Potato	Canada	0
Potato	Maine	0
Tomato	Eden, New York	74
Salvia	Eden, New York	78
Bamboo	Bermuda	0
Potato	Minnesota	0
Potato	Williamson, New York	0
Eggplant	Eden, New York	98
Unknown	Williamson, New York	0
Grass	Massachusetts	0
Unknown	Unknown	60
Pepper	Albany, New York	97
Cabbage	Eden, New York	98
Checks		0

added to the soil immediately surrounding the seed piece and a little above the upper surface of the tuber, so that any sprouts would pass directly through it. The pot was filled with the remaining soil. All the pots were kept in the same greenhouse where the previous experiment had been conducted. This house was comparatively cool, having a temperature of approximately 8° to 15° C. at night, and about 10° to 18° C. during the day. The soil was kept moderately moist, but no attempt was made to control accurately the moisture content. All the tubers sprouted. When they were from two to three inches high, many were removed from the soil and examined, but no lesions were observed on the stems. The plants were carefully replaced and allowed to grow for two and one-half months. At the end of that time they were removed from the soil, and the stems and rootlets were examined for lesions, and the new tubers and the seed pieces for sclerotia.

Parallel with this experiment, fifty tubers were placed in soil infected with the cabbage strain of the fungus. When the sprouts were two or three inches high, and no lesions were developing, ten plants were reinoculated just beneath the surface of the ground and these were kept in a moist chamber for forty-eight hours. They were then removed to the greenhouse bench. A little later, when the plants were from six to eight inches high, cabbage seed was sown around the base of twenty-five of the plants growing in the soil infected with the fungus from cabbage.

A large proportion of these seedlings developed typical wire stem. This demonstrated that the fungus was still in the soil and in an active condition. The tubers were harvested when they were from one to two inches in diameter. Very few definite lesions were produced even by the strains from potatoes, probably because of the unfavorably low temperature. The striking difference was in the occurrence of the sclerotia on both the old seed pieces and the new tubers, many sclerotia having been produced by the strains from potatoes, but none by the cabbage strain in any instance (table 8). None of the lesions reported in the table were very large, although all were typical. It is to be noted that every potato strain produced lesions, even though few in number, while only four of the other strains did so. Only the potato strains produced sclerotia on the new tubers and on the roots. With one or two exceptions the potato strains produced the most and the largest sclerotia on the cut surfaces of the seed pieces. The cabbage, tomato, grass, bamboo, and lettuce strains produced no signs nor symptoms of the disease on either the roots, the stems, or the tubers.

TABLE 8. RESULTS OF INOCULATING POTATOES WITH DIFFERENT STRAINS OF CORTICIUM VAGUM

Number of pots	Strains	Lesions		Sclerotia *			Number of new tubers
		Roots	Stems	Roots	Seed pieces	Tubers	
10	Unknown	0	0	0	0	0	14
10	Potato	0	2	++	++++	++++	16†
10	Salvia	0	2	0	+++	0	14
10	Potato	2	0	0	0	0	19
10	Lettuce	0	0	0	0	0	12
10	Pepper	0	2	0	0	0	18
10	Potato	5	0	+	+++	+++	13†
10	Eggplant	2	0	0	+	0	15
10	Pea	3	0	0	+	0	12
10	Bamboo	0	0	0	0	0	15
10	Potato	0	2	0	+	+	15
10	Tomato	0	0	0	0	0	19
10	Unknown	0	0	0	+	0	15
10	Unknown	0	0	0	+++	0	16
10	Grass	0	0	0	0	0	15
40	Cabbage	0	0	0	0	0	100
10	Cabbage (stems inoculated twice)	0	0	0	0	0	25
10	Controls	0	0	0	0	0	21

* +, an occasional sclerotium, or very slight lesion; ++, few sclerotia, or rather definite lesions; +++, numerous sclerotia, or deep lesions; ++++, very many sclerotia, or several deep lesions.

† Stems of most of the tubers were covered with the weblike mycelial mat characteristic of *Corticium vagum*.

Two strains from potatoes (indicated by daggers in table 8) produced definite dark brown wefts of mycelium on the stems of the plants. Some of the pots were placed in a moist chamber, and some under bell glasses, with the hope that the perfect stage might be obtained, but these efforts were unsuccessful. It is to be noted (table 8) that these two strains produced definite lesions on either the stem or the roots, and also produced the most and the largest sclerotia on both the old seed pieces and the new tubers. It is worthy of note that of these two strains, the one producing the most lesions and sclerotia was the one which was isolated from the potatoes from Canada two years earlier and at that time demonstrated to be non-pathogenic on cabbage (page 19). Here, then, it is to be observed that the pathogenic cabbage strains produce neither lesions nor sclerotia on any part of the potato, but it is possible to get infection with them on cabbage under a wide range of conditions; also, that the strains from potatoes, non-pathogenic on cabbage, produce definite lesions and sclerotia in numbers on their own host.

Extensive inoculation experiments were next undertaken with freshly isolated potato strains to determine conclusively whether the differences in pathogenicity of the various strains were due to a specific relationship between host and parasite, or to other factors. From forty to fifty seeds of cabbage were sown in sterile soil in 4-inch pots. When the seedlings were two inches high, inoculations were made with forty-one freshly isolated strains from potatoes. Thirty-nine of these were obtained from sclerotia from five different lots of tubers from three different counties in New York, and two from different lots of southern potatoes bought at a retail store. Two pots were used for each strain. This experiment was repeated later in duplicate, as before, with twenty-two of the strains. Ten pots were reserved as checks in both trials. Every seedling was observed, and eight slightly discolored stems were found in the first trial. This, however, was a discoloration only and not a case of wire stem or damping-off. In the second trial, all of the seedlings in one pot damped-off, as did also five others in each of two other pots. This damping-off was evidently a result of improper sterilization of the soil, since no diseased plants were produced in any of the duplicates in the above series, and since twelve pots of seedlings had to be discarded before the inoculations were made. The seedlings in five of the controls developed the disease later. The plants in four pots in each of these trials were inoculated with the cabbage strain, and practically 100 per cent of infection resulted in every case. All of the checks remained healthy except where the soil was not properly sterilized. From these data, where from 35 to 50 seedlings were inoculated in each of 125 pots, or a total of from 4500 to 5000, with negative results, the conclusion is warranted that the freshly isolated potato strains are not pathogenic on cabbage.

Parallel with the preceding experiment, a strain of *Corticium vagum* isolated from asters in December, 1922, at the college greenhouse, was used to inoculate cabbage seedlings at different times. Negative results were consistently obtained. The cabbage strain under the same conditions produced 100 per cent of damping-off, and the checks remained healthy. To further test this particular strain, seedlings were inoculated as follows: two rows on one end of a flat were inoculated with the cabbage strain, and two rows on the other end with the aster strain. The three rows at the center served as controls. The center rows and those inoculated with the aster strain remained healthy. Of the plants inoculated with the cabbage strain, 80 per cent damped-off. Therefore the aster strain tested here is not pathogenic on cabbage. Inoculations on asters at this time demonstrated that the strain is pathogenic on that host.

In a study of the question as to whether or not the strains freshly isolated from potato tubers are pathogenic on potatoes, the following experiments were performed. Tubers of the Bliss Triumph variety, treated with corrosive sublimate as before, were planted in the temperature tanks (page 26,) where both the temperature and the moisture of the soil could be controlled. Richards' (1921, 1923) strain of *Corticium vagum* could infect potatoes at a wide range of temperature, 9° to 27° C., with the optimum near 18°. The optimum for the host was about 24° C. In view of these facts, the soil temperature was maintained at 22° in order to hasten germination but still remain within the range of infection. The moisture content of the soil was maintained at about two-thirds of its water-holding capacity. Sterilized soil in glazed earthenware gallon jars, measuring 19 by 19 centimeters, was used in all cases. One seed piece was placed in each jar, and five jars were used for each strain and twenty-five jars for each tank. One set of five jars was inoculated with the cabbage strain as a check on the previous experiment (table 8). The inoculum was placed around the seed piece and in the same plane as the upper surface of the tuber, to insure the contact of the sprout with the fungus. When the plants were three inches high, 70 per cent of all the stems inoculated with the potato strains had distinct lesions. All the strains tested were pathogenic, each producing lesions on from two to five of the five stems inoculated. Wounding and reinoculating the uninjured stems increased the total number of injured stems to 80 per cent. The initial inoculation with the cabbage strain gave negative results, and a reinoculation after wounding produced slight discoloration on three stems. It is not positive that this discoloration was caused by the fungus, as these lesions became no more pronounced after ten days. In a second tank, maintained at exactly the same temperature and moisture conditions, twenty-five tubers were inoculated with the cabbage strain as before. Here three stems developed small, superficial, apparently positive lesions, but these failed to develop further. All the stems, except the three mentioned, were wounded and a large amount of inoculum was pressed tightly against the wound, but no further lesions developed. Again, it is doubtful whether the cabbage strain caused these lesions. In a third tank, where the soil temperature fluctuated between 15° and 19° C., twenty seed pieces were inoculated with potato strains and five with the cabbage strain. The sprouting of the tubers, as compared with those in the other tanks, was delayed a week or ten days because of the low temperature. For the same reason, the initial inoculation produced infection on only 25 per cent of the stems. Various methods of reinoculation were attempted, resulting in lesions on 65 per cent of the stems. No lesions were produced by the cabbage strain. In this trial many sclerotia were produced on the rootlets and even on the stems. Both the potato and the cabbage strains produced sclerotia in abundance on their respective hosts, but were never observed to do so on the opposite host. When the cabbage seedlings were inoculated with the potato strains, the soil and the base of the stems were frequently covered with the fungus mycelium, but neither sclerotia nor lesions were observed.

A total of 230 inoculations of potatoes were made with thirty-two strains of the fungus freshly isolated from potatoes, and nineteen of these strains were definitely pathogenic. The remaining thirteen likewise might have proved pathogenic had the temperature been more favorable. These negative results were obtained when the soil temperature fluctuated between 22° and 25° C., a temperature too high for the rapid development of the disease (Richards, 1923). This was demonstrated by inoculating potato stems at these higher temperatures with strains previously proved to be pathogenic. Under such conditions these likewise produced no lesions. Parallel with the above-mentioned inoculation experiments, sixty-five tubers were inoculated with the cabbage strain and placed under these controlled conditions. No definite lesions developed, demonstrating the non-pathogenicity of this strain on potatoes. In the light of the hundreds of inoculations on cabbage seedlings with the pathogenic strains isolated from potatoes, and the large number of inoculations on potatoes with the cabbage strain, all under more or less favorable conditions, it must be concluded that a specific pathogenicity exists.

Longevity of the fungus in the soil

Little is known concerning the actual length of time that this fungus will persist in the soil. Since it is parasitic on a considerable number of plants, the possibility is that it may live in the soil indefinitely. Further, the organism is saprophytic on the organic matter in the soil (Rolfs, 1902, 1904), and the richer the soil is in humus, the more favorable are the conditions for its development. When the investigations on this problem were discontinued at the approach of the summer season in 1921, the pots containing the fungus-infected soil were placed under the greenhouse benches and permitted to completely air-dry. The following fall this soil was thoroughly mixed and cabbage seed was planted in it. The same procedure was followed also with the soil that was used the second year. In both seasons the percentages of diseased plants were approximately as great as from naturally infected soil or from soil into which had been mixed an average amount of inoculum. The soil was left in this condition for about seven months. These conditions were probably more unfavorable than any field conditions would be, since no plants were growing in the soil, the temperature was abnormally high in the greenhouse, and the soil was practically free from moisture.

TEMPERATURE AND MOISTURE STUDIES

It is evident from the literature that temperature and moisture are important factors in the parasitism of this fungus. Johnson (1914), Peltier (1916), Sherbakoff (1917, b), and others suggest that damping-off in general is favored by wet soils and humid atmospheres, relatively high temperatures, and poor ventilation. Rolfs (1904) concluded from his laboratory investigations that a high temperature and plenty of moisture were essential for the development of *Corticium vagum.* He suggested that this may be the reason why heavily watered fields of potatoes may be severely injured in hot weather, while those sparingly watered and well cultivated are apparently not injured. Richards (1921, 1923) obtained infection within a temperature range of 9° to 27° C., with the optimum approximately at 18°. Considerable infection, however, was obtained at from 15° to 21°, depending on slight changes in environmental conditions.

Experimental methods and data

Temperature-moisture relations

Since no report was to be found in literature of researches to determine definitely the interrelationship of soil moistures, soil temperatures, and damping-off by *Corticium vagum*, some experiments in this connection were performed. The results of preliminary experiments were in accordance with the conclusions of Johnson (1914) and others who found that a combination of high soil moisture, high humidity, and poor ventilation, is favorable for the development of fungi which cause damping-off.

This phase of the problem was studied in controlled-temperature tanks. These tanks, as used by Burkholder,[11] were 4 feet square and 8 inches deep, and contained water the temperature of which was automatically controlled by electrical units. In the experiments conducted by the writer, a third tank, not electrically controlled, was thoroughly insulated with sawdust and cork and was used for the lower temperatures only. Several changes of cold water during the day aided in maintaining the desired temperature in this tank. The surface of all the tanks was covered with paraffined canvas, into which sixteen holes were cut at definite intervals to permit the immersion of ordinary glazed gallon jars (19 by 19 centimeters).

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[11] Burkholder, Walter H. The effect of two soil temperatures on the yield and water relations of healthy and diseased bean plants. Ecology 1:113–123. 1920.

With this arrangement and good thermostats, it was possible to keep the temperature of the water bath constant so that an ordinary thermometer registered no variation for weeks at a time (figure 9, page 41). The temperature in the uncontrolled tank, if adjusted two or three times a day, would fluctuate within two or three degrees. Thermographs were installed in each tank. These, though fairly accurate, required considerable adjusting, and were not relied upon for temperature readings but were used to indicate changes in temperature only. The temperature of both the water bath and the soil in the culture vessels, as well as that of the air, was taken several times daily by means of thermometers. While the temperature of the water remained constant, it is evident that in an ordinary greenhouse the soil temperature nevertheless varies, depending on the intensity of the sunlight and the fluctuations of the air temperature. The greater the difference between the air temperature and the water temperature, the greater are the fluctuations in the soil temperature and the greater is the difference between the soil and the water temperature. For example, when it was desired to maintain the soil at 32° C., it was necessary to maintain the water at 35°, since the house was kept at approximately 10° to 15° C. at night and at about five to ten degrees warmer during the day. However, the air temperature was extremely variable, and bright sunlight would cause the soil in this tank to reach a temperature somewhere between 33° and 34° C. As soon as the direct rays of the sun were no longer on the culture vessels, the temperature would drop rapidly to approximately 32°. The limits were, however, never higher than as indicated in the tables, and usually were lower. The temperatures given in the tables are the maxima and minima taken in the upper half-inch of the soil, as this is the region most directly related to damping-off. Various measures, such as maintaining the surface of the soil an inch or more below the surface of the water, and planting the seedlings close to the sides of the jar, were taken to aid in keeping the temperature constant. With three tanks, three temperatures could be run simultaneously.

The moisture content of the soil, in all of these trials, was also controlled. Livingston auto-irrigators[12] were used, two in each jar. The maximum water-holding capacity of the soil was determined by the Hilgard 1-centimeter-column method.[13] Two series of moistures were maintained throughout the trials. In order to keep the moisture content of each culture vessel comparable with the others in any one moisture series, half of the culture vessels in one tank were connected with half of those in each of the other tanks, with but one intake valve for that series. The other halves of the jars in the respective tanks were similarly connected, and constituted the second series. The weak point in the system was the mercury valve. The pull increased as the plants became larger, and occasionally this resulted in the pulling-over of the mercury into the cup. In order not to have the pots flooded, the intake was placed in a

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[12] Livingston, B. E. A method for controlling plant moisture. Plant world **11**:39–40. 1908.

Hawkins, Lon A. The porous clay cup for automatic watering of plants. Plant world **13**:220–227. 1910.

Livingston, B. E., and Hawkins, Lon A. The water-relation between plant and soil. Carnegie Inst. Wash. Publ. 204:3–48. 1915.

[13] Hilgard, E. W. Soils, p. 209. 1911.

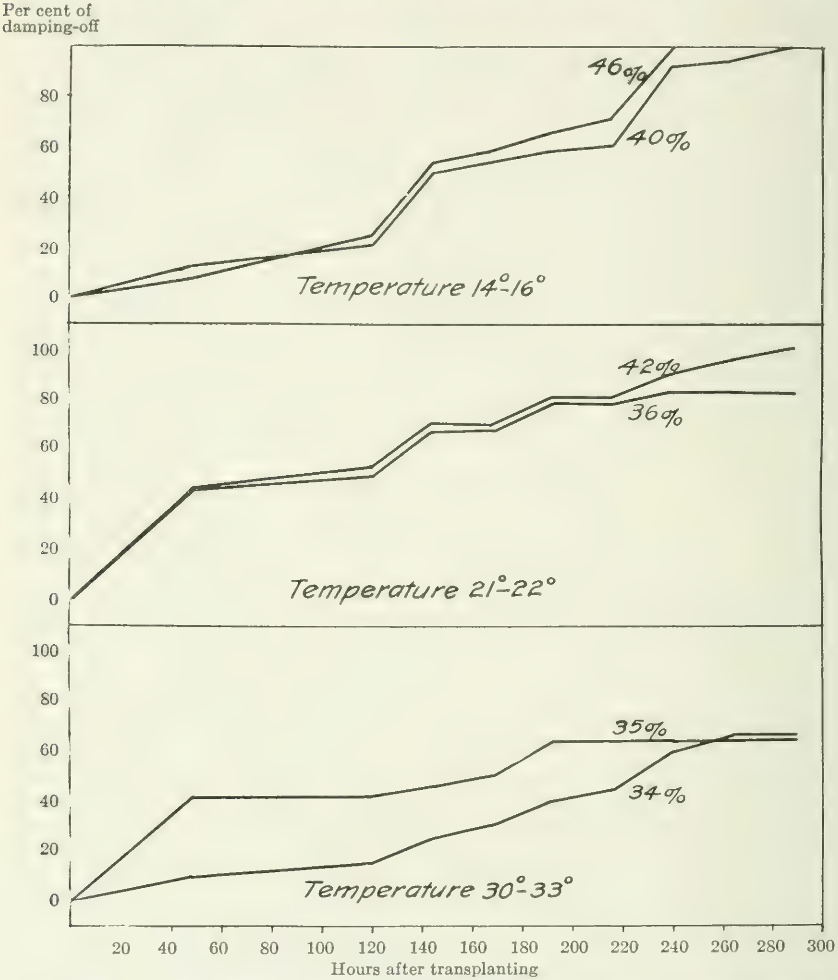

FIGURE 1. INFLUENCE OF DIFFERENT SOIL TEMPERATURES AND SOIL MOISTURES ON RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS CAUSED BY CORTICIUM VAGUM (TABLE 9, TRIAL I)

Soil moistures are given as percentages

500-cubic-centimeter bottle. This eliminated the danger of flooding, and in addition supplied a means by which the working of the system could be observed. It is obvious that after the water content had been adjusted in each system, each would require about the same amount of water,

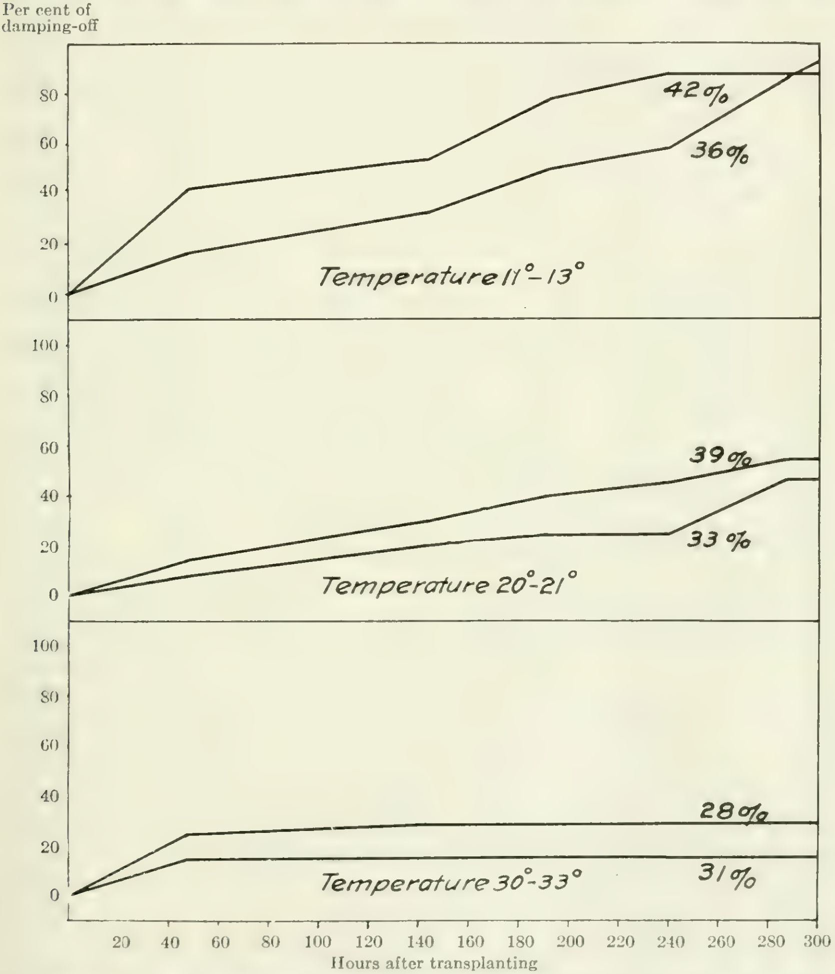

FIGURE 2. INFLUENCE OF DIFFERENT SOIL TEMPERATURES AND SOIL MOISTURES ON RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS CAUSED BY CORTICIUM VAGUM (TABLE 9, TRIAL II)

Soil moistures are given as percentages

since there was the same amount of soil in the two systems. While results were obtained, this method of moisture control is too cumbersome to be used on a large scale.

The soil was sterilized by autoclaving for from two to three hours under 12 to 18 pounds pressure. The inoculum was mixed into the soil, the system was set up, and the moistures and temperatures were adjusted. Four seedlings were transplanted into each culture vessel. The seedlings were transplanted rather than started in the vessels, because if the seed had been sown directly the seedlings would have been attacked by the fungus before they could push through the soil. Furthermore, several weeks could be gained in this way. This method, however, presented several disadvantages. First, the transplanting was likely to be a definite shock to the plant, and secondly, with the fungus directly in the soil it was impossible to know whether the plant was directly in contact with the inoculum or some distance away from it. Where the difference in the rate as well as the total amount of damping-off was to be compared at the various temperatures and moistures, this represented a large source of error. Also, when the plants were transplanted into the infected soil, a small amount of water had to be given them to insure growth, and this favored the growth of the fungus about the roots. Four jars in each tank were kept as controls. The moisture content was determined after the conclusion of several experiments to insure the uniform operation of the systems for each of the experiments.

During the winter of 1921–22, six trials were made with a total of eighteen temperatures (table 9). In general, the results were similar for all six trials. Damping-off occurred at all of the temperatures tried, but the rate decreased above 30° C. Low temperatures and low moistures were unfavorable for the development of the disease. Determinations made at the end of the trials showed that slightly different moistures resulted at the different temperatures. This was probably due to a more rapid rate of evaporation and transpiration at the higher temperatures. Moisture determinations were made at the conclusion of trials I, II, and VI. In trial VI both irrigation systems became clogged, resulting in a lower moisture content of the soil in both series. For trial IV another set of plants was transplanted into the soil used in the previous experiment without disturbing it any more than was necessary. In this trial, tank 3, which was held at 32° C. in the previous experiments, was lowered to 19.5° C. There was a definite decrease in the rate of damping-off. Whether or not this was due to a decrease in the virulence of the fungus because of the previous high temperatures was not ascertained. For trials V and VI, the upper three inches of the soil was removed from the jars, thoroughly mixed to insure equal distribution of the inoculum, and replaced.

TABLE 9. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON WIRE STEM OF CABBAGE CAUSED BY CORTICIUM VAGUM B. & C.

Number of trial	Number of tank	Soil		Percentage of damping-off at indicated hours after transplanting											Condition of plants†			
		Temperature (C.)	Moisture (per cent)*	Hours											I	II	III	IV
				48	120	144	168	192	216	240	264	288	312	384				
				Per cent of damping-off														
I	1	14°–16° (Mode 15.5°)	46	8	25	54	58	66	72	100	100	100	100	100	0	5	50	45
			40	13	22	50	54	58	60	92	94	100	100	100	0	8	54	38
	2	21°–22°	42	45	53	70	70	81	81	90	95	100	100	100	0	10	20	70
			36	45	50	67	67	79	79	83	83	83	83	83	12	42	17	29
	3	30°–33° (Mode 30.0°)	35	42	42	46	50	64	64	64	64	64	64	64	20	40	25	15
			34	10	15	25	30	40	45	60	65	65	65	65	25	21	8	46
II	1	11°–13° (Mode 12.5°).	42	42	....	54	....	79	....	88	....	88	100	100	0	17	58	25
			36	17	....	33	....	50	....	58	....	88	88	88	12	4	75	9
	2	20°–21°	39	15	....	30	....	40	....	45	....	55	55	55	45	5	30	20
			33	8	....	20	....	25	....	25	....	47	47	47	62	13	4	21
	3	30°–33° (Mode 31°)	31	15	....	15	....	15	....	15	....	15	15	15	90	0	0	10
			28	25	....	29	....	29	....	29	....	29	29	29	75	4	0	21
III	1	10°–13° (Mode 12°)	42	4	33	33	66	75	83	83	87	87	91	91	9	4	87	0
			36	8	16	29	42	46	54	54	63	63	63	63	17	13	42	28
	2	18°–19° (Mode 18.5°)	39	35	65	75	85	90	90	90	90	90	90	90	5	5	55	35
			33	30	37	50	50	54	62	62	63	63	63	63	21	21	21	33
	3	31°–34°. (Mode 31°)	31	20	30	35	40	40	44	44	44	44	44	44	45	15	10	30
			28	25	30	33	37	38	41	42	42	42	42	42	42	33	0	25

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* Percentage of maximum retentive power of soil as determined by the Hilgard method.
† I, healthy; II, slightly diseased; III, badly diseased; IV, dead.

TABLE 9 *(concluded)*

Number of trial	Number of tank	Soil		Percentage of damping-off at indicated hours after transplanting											Condition of plants †			
		Temperature (C.)	Moisture (per cent) *	Hours											I	II	III	IV
				48	120	144	168	192	216	240	264	288	312	384				
				Per cent of damping-off														
IV	1	10°–12° (Mode 11°)	42 36	54 8	62 8	75 8	75 16	79 16	79 25	87 37	87 38	91 42	92 46	92 46	4 33	8 13	75 50	13 4
	2	24°–25° (Mode 24.5°)	39 33	35 12	45 25	60 25	65 25	65 29	70 33	80 33	90 33	90 37	90 37	90 37	10 45	15 17	50 25	25 13
	3	19°–20° (Mode 19.5°)	39 33	15 8	30 16	30 20	30 21	35 21	35 21	35 21	60 25	70 25	70 25	70 25	25 58	30 8	35 21	10 13
V	1	12°–13° (Mode 12.5°)	42 36	33 4	46 12	50 12	63 16	66 21	79 25	83 37	92 37	92 42	92 42	92 42	0 58	4 13	83 21	13 8
	2	18°–19° (Mode 18.5°)	39 33	30 4	50 12	60 29	60 33	70 41	80 50	80 54	80 54	85 58	85 62	85 62	5 50	25 13	65 37	10 0
	3	30°–32° (Mode 31.5°)	31 28	5 4	5 4	5 8	10 12	10 12	10 16	15 16	25 16	25 16	25 16	25 16	60 87	5 0	30 9	5 4
VI	1	14°–18° (Mode 16.5°)	36 32	0 0	0 0	4 4	17 17	17 17	25 29	33 37	38 42	38 42	38 42	38 42	42 50	8 12	46 34	4 4
	2	22°–24° (Mode 22.5°)	30 27	0 0	0 0	4 4	13 13	17 17	25 17	25 21	25 21	29 21	29 21	29 21	60 66	30 13	10 8	0 13
	3	25°–27° (Mode 25.5°)	28 26	0 0	0 0	0 0	4 0	8 0	10 0	12 0	13 4	13 4	13 4	13 4	80 83	10 4	10 13	0 0

* Percentage of maximum retentive power of soil as determined by the Hilgard method.

† I, healthy; II, slightly diseased; III, badly diseased; IV, dead.

These experiments were repeated the following year with a reduction in the sources of error encountered in the first year. A complete new system was set up for each trial. First, the gallon jars were replaced by others of the same diameter but not so high (13 by 19 centimeters). These

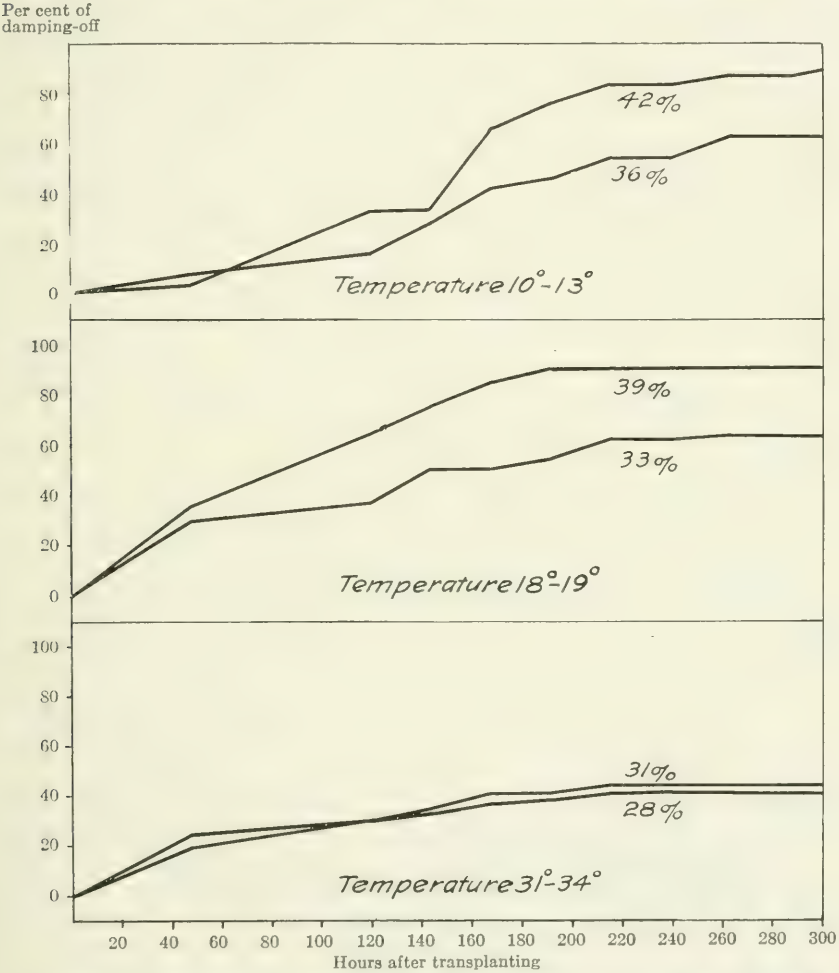

Figure 3. INFLUENCE OF DIFFERENT SOIL TEMPERATURES AND SOIL MOISTURES ON RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS CAUSED BY CORTICIUM VAGUM (TABLE 9, TRIAL III)

Soil moistures are given as percentages

could be elevated from the bottom of the tank by placing them on small inverted flowerpots. Better circulation in the water bath was thus afforded, so that a larger number of culture vessels could be placed in each tank without reducing the uniformity of the temperature. Instead

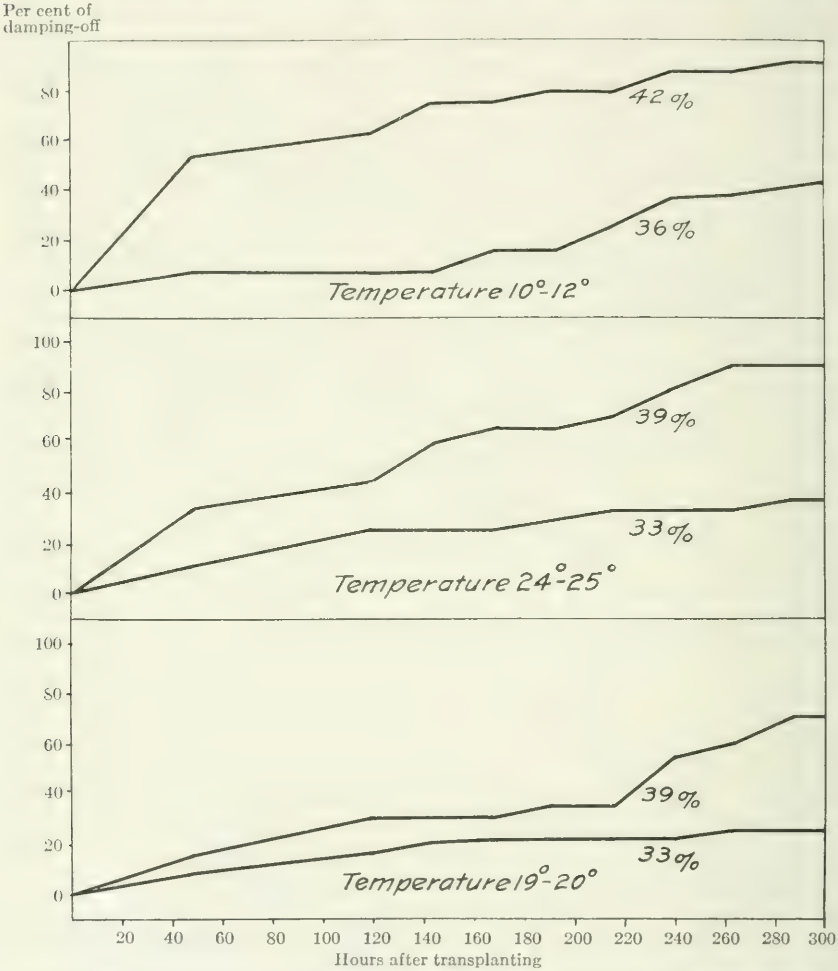

FIGURE 4. INFLUENCE OF DIFFERENT SOIL TEMPERATURES AND SOIL MOISTURES ON RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS CAUSED BY CORTICIUM VAGUM (TABLE 9, TRIAL IV)

Soil temperatures are given as percentages of only sixteen culture vessels, twenty-five were now used. The same amount, by weight, of well-screened, sterilized soil was placed in each vessel, and provision was made for watering from the bottom by inserting a glass tube which connected with an inverted 2-inch flowerpot at the

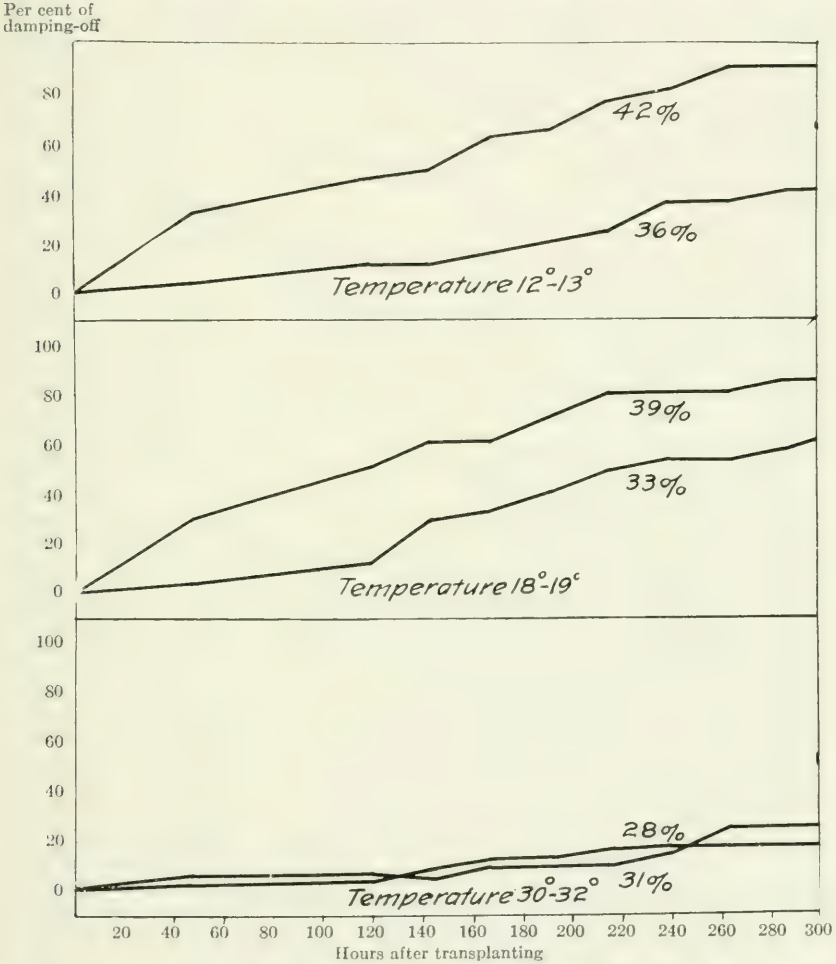

FIGURE 5. INFLUENCE OF DIFFERENT SOIL TEMPERATURES AND SOIL MOISTURES ON RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS CAUSED BY CORTICIUM VAGUM (TABLE 9, TRIAL V)

Soil moistures are given as percentages

bottom of the soil. The calculated amount of water necessary to produce the desired moisture content of the soil was added through this tube. The maximum retentive power and the actual moisture of the soil were determined previous to the placing of the soil in the jars.

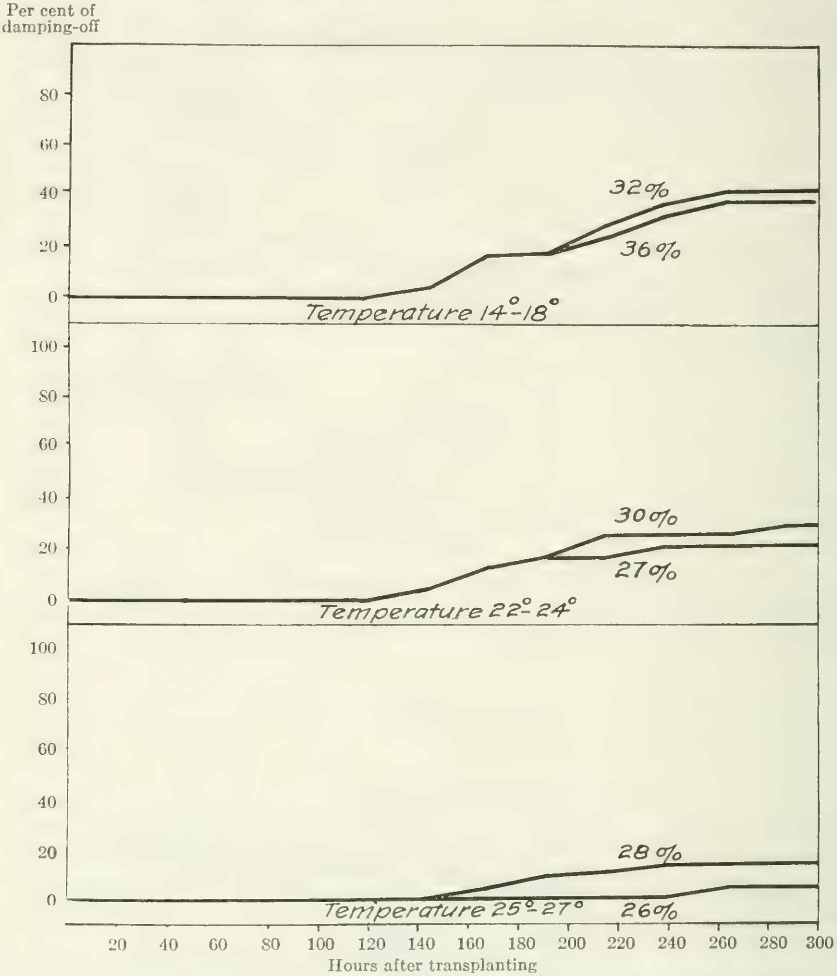

FIGURE 6. INFLUENCE OF DIFFERENT SOIL TEMPERATURES AND SOIL MOISTURES ON RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS CAUSED BY CORTICIUM VAGUM (TABLE 9, TRIAL VI)

Soil moistures are given as percentages

The tared culture vessels were maintained at constant weight by weighing every day or two, depending on the rate of evaporation and transpiration, and adding the necessary water. Five moisture series were maintained for each tank, with five culture vessels for each series. By this method the moistures in each series in each tank were comparable to their respective series in the other tanks maintained at different temperatures. One jar in each moisture series was kept as a control. Instead of having only four plants for each culture, from twelve to fifteen seedlings were transplanted into each vessel. Thus from forty-eight to sixty plants were growing at each moisture series at each of the three temperatures, not including the culture vessel with the fifteen control plants. The seedlings were allowed to become established (six to eight days) before inoculation. The method of inoculation consisted in placing a grain of wheat, upon which the fungus was growing, about one-quarter inch below the surface of the soil and about that distance from the stem. This permitted the fungus to grow through the soil to the seedling. Several cubic centimeters of water was added to the seedlings at the time of transplanting, to insure their growth. Probably very little of this moisture remained at the base of the seedlings at the time of inoculation. Obviously, since gravity limits the capillary ascent in a progressive ratio,[14] the surface of the soil must have been considerably drier than the bottom layers, and the figures given in the tables are but an approximation for the entire culture vessel, and not for the surface layer, where damping-off actually takes place. It is further to be pointed out that since the Hilgard 1-centimeter-column method is a laboratory procedure and the soil used is not in its normal structural state, figures thus obtained may be from 30 to 130 per cent too high.[15] To determine the amount of water actually required to saturate a column of soil as high as the column used in the culture vessels, a tin pail, of the same size as the jars and with the bottom perforated, was filled with soil to the same height as that in the culture vessels and then set in water, which was allowed to seep through the perforations. After this had drained for a short time, the weight of water taken up was determined. Numerous determinations of this kind indicated that the error introduced by the centimeter-cup method was approximately 70 per cent. The maximum amount of water required to saturate the soil as determined by the Hilgard 1-centimeter-column method was 85 per cent of the dry weight, while but 51 per cent saturated a column of soil as high as that used in the culture vessels. In the tables the moisture contents are expressed as determined by the standard Hilgard method.

The results are tabulated in tables 10 to 15. Inspection of the tables shows that the results of these trials, in the main, agree with those of the preceding year. It is not necessary to discuss each of the experiments in detail, except to point out briefly some of the variations in the method. Similar moisture series were maintained in each of the experiments, except in the first of these trials (table 10), in which the highest moisture content was greater than 51 per cent (saturation). The other differences

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[14] Hilgard, E. W. Soils, p. 208–209. 1911.
[15] Lyon, T. Lyttleton, and Buckman, Harry O. The nature and properties of soils, p. 162–163. 1922.

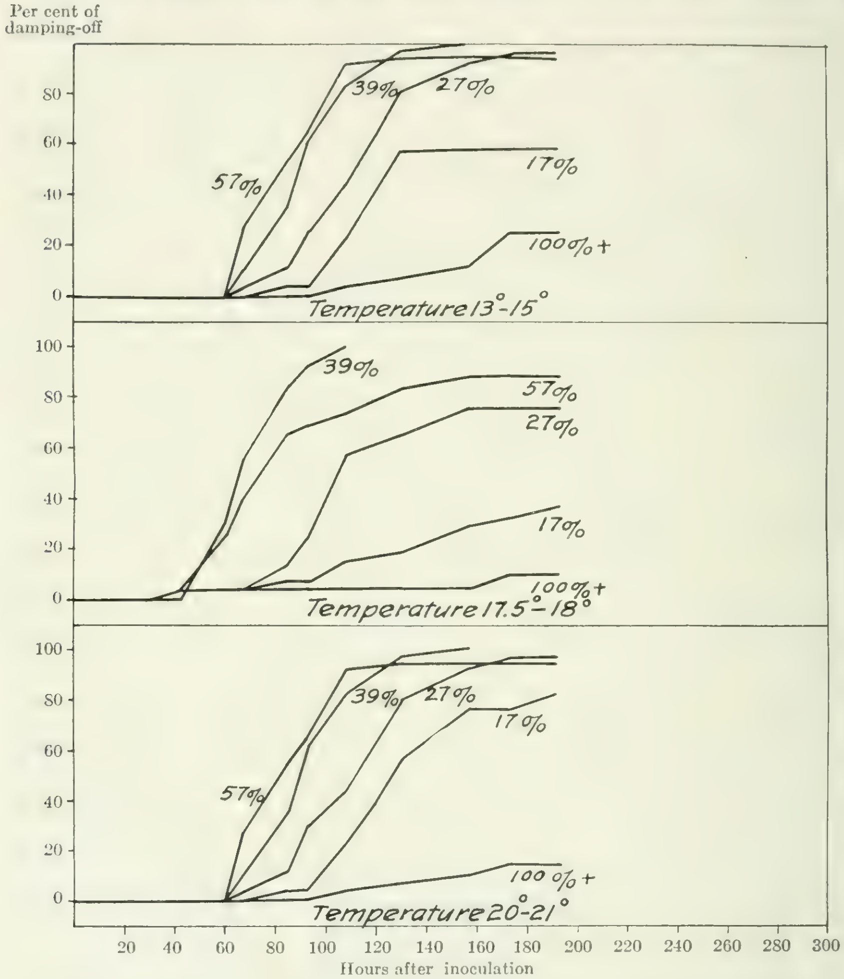

FIGURE 7. INFLUENCE OF DIFFERENT SOIL TEMPERATURES AND SOIL MOISTURES ON RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS CAUSED BY CORTICIUM VAGUM (TABLE 10, TRIAL VII)

Soil moistures are given as percentages] in the moisture content were slight and did not alter the results, as will be seen when they are compared with those in the other trials. In all the trials except VII and VIII (tables 10 and 11), a moisture content of 18 per cent was too low to support growth. Apparently the soil in trial

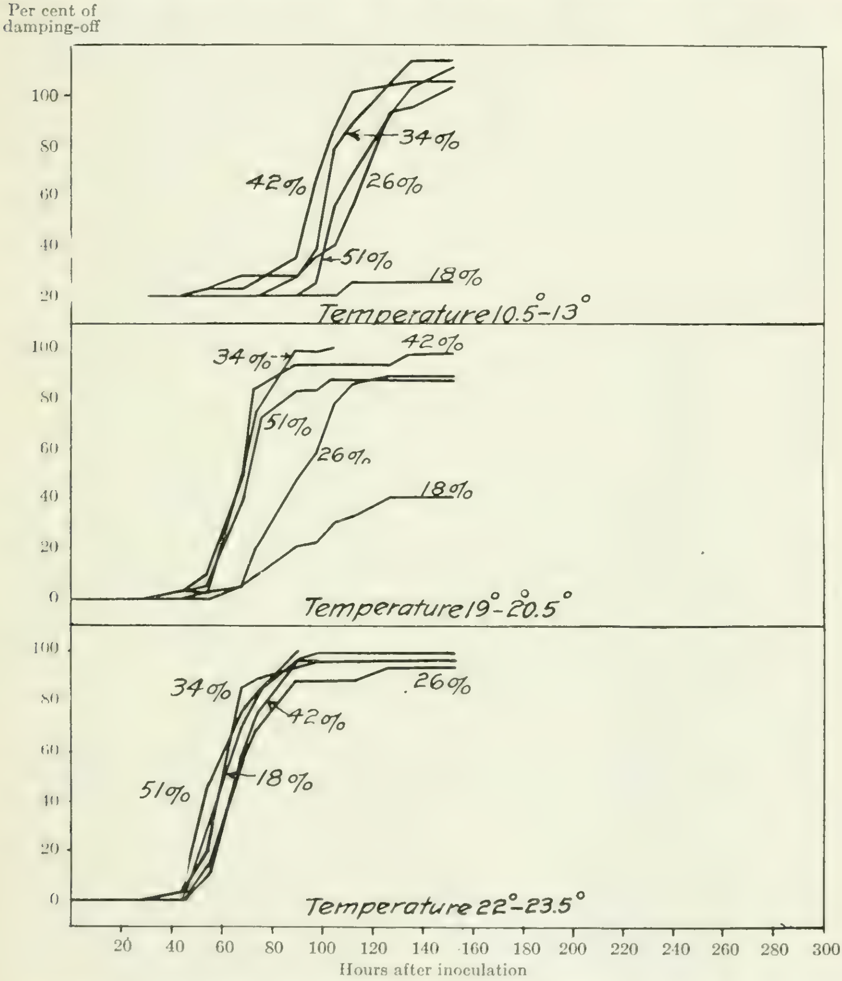

FIGURE 8. INFLUENCE OF DIFFERENT SOIL TEMPERATURES AND SOIL MOISTURES ON RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS CAUSED BY CORTICIUM VAGUM (TABLE 11, TRIAL VIII)

Soil moistures are given as percentages

VII was of slightly different consistency, as only incipient wilting was observed in the soil with a moisture content of 17 per cent, while in the other trials permanent wilting resulted at this low moisture content. In trial VIII, tank 3 (table 11), the greatest rate of damping-off was observed in the soil where the moisture content was 18 per cent. This was due to the fact that the plants had started to wilt and were given a small amount of water several days previous to inoculation. The total amount of moisture in the culture vessel was not in excess of the required amount, but the immediate area surrounding the seedlings had an excess as compared with the previous experiments. This was a favorable condition for fungus growth. In most of the experiments a soil with a moisture content of 51 per cent caused a delay in the rate of damping-off. This was almost at saturation and a detrimental effect on the host was also noted. In trial X (table 13) an error was made in the original calculation, and consequently two moistures were obtained where permanent wilting occurred. The redeterminations at the end of the experiment indicated just what these moistures were. In trials X and XI (tables 13 and 14), the low temperature was maintained by packing snow in one end of the tank. The heater was also in operation to prevent any undercooling. The uniformity of temperatures maintained is indicated in figure 9.

In all the preceding experiments, none of the check plants developed the disease.

Good growth of the host was observed at all temperatures between 15° and 30° C. Temperatures below 15° slightly decreased the rate of growth, but it was only at the lowest temperature (5.5° C.) that the plants made very little growth. Even at that temperature the plants were in a very thrifty condition at the end of the experiment. Above 30° C. the growth was rapid, but the plants were not so intensely green in color as at the lower temperatures, and they showed clearly the effects of the abnormal environmental conditions.

The following conclusions are to be drawn from these temperature-moisture experiments:

1. A temperature of approximately 7° or 8° C. is the minimum for damping-off of cabbage seedlings caused by *Corticium vagum*, as a small number of diseased plants were obtained at 10° C. and none at approximately 5.5° C. (tables 13 and 14).

2. A temperature of 31° to 32° C. is the maximum for the destructive action of the fungus (tables 9, 13, and 15).

3. There is no sharply defined optimum, but a rapid rate of damping-off may occur at any temperature from 15° C. to 30° C. There is a decrease from these temperatures until the respective limits are reached (tables 9 to 15).

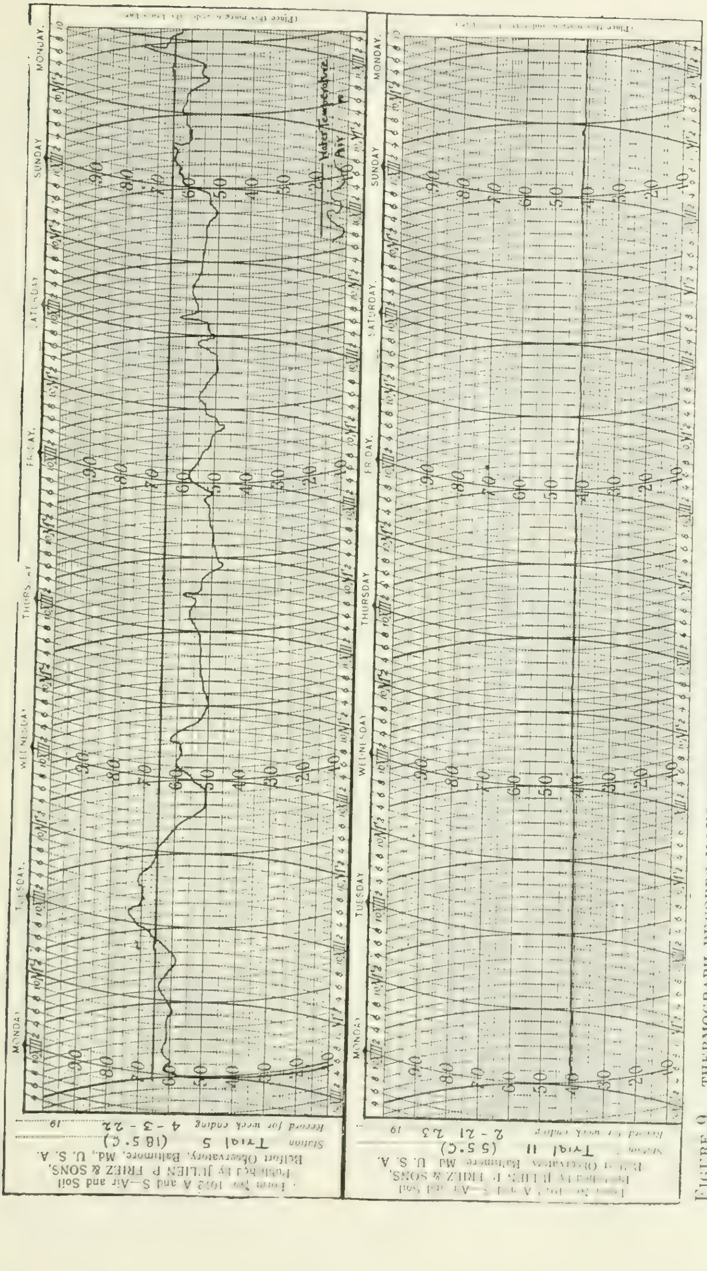

FIGURE 9. THERMOGRAPH RECORDS ILLUSTRATING THE UNIFORMITY OF BOTH LOWER AND HIGHER TEMPERATURES MAINTAINED IN TANKS DURING INVESTIGATIONS OF WIRE STEM OF CABBAGE

TABLE 10. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON WIRE STEM OF CABBAGE CAUSED BY CORTICIUM VAGUM B. & C.

Number of trial	Number of tank	Soil		Percentage of damping-off at indicated hours after inoculation											Condition of plants†			
		Temperature (C.)	Moisture (per cent)*	Hours														
				30	42	60	67	85	93	108	130	157	173	192	I	II	III	IV
				Per cent of damping-off														
VII	1	13°–15° (Mode 14.8°)	17	0	0	0	0	4	4	23	57	58	58	58	42	0	58	0
			27	0	0	0	4	11	26	44	81	93	96	96	4	8	81	7
			39	0	0	0	11	36	61	83	97	100	100	100	0	0	60	40
			57	0	0	0	27	54	65	92	95	95	95	95	5	3	69	23
			100+	0	0	0	0	0	0	4	7	11	25	25	75	0	0	25
	2	17.5°–18° (Mode 17.5°)	17	0	4	4	4	7	7	15	19	30	33	37	63	16	14	7
			27	0	4	4	4	14	25	58	65	76	76	76	24	8	57	11
			39	0	0	30	56	85	93	100	100	100	100	100	0	0	68	32
			57	0	0	27	38	65	69	73	84	88	88	88	12	11	63	14
			100+	0	4	4	4	4	4	4	4	4	10	10	90	6	0	4
	3	20°–21° (Mode 20.0°)	17	0	0	0	0	4	4	23	57	76	76	82	18	0	62	20
			27	0	0	0	4	11	30	44	81	93	96	96	4	0	18	78
			39	0	0	0	11	36	61	83	97	100	100	100	0	0	30	70
			57	0	0	0	27	54	65	92	95	95	95	95	5	0	70	25
			100+	0	0	0	0	0	0	4	7	10	14	14	86	14	0	0

* Percentage of maximum retentive power of soil as determined by the Hilgard method.

† I, healthy; II, slightly diseased; III, badly diseased; IV, dead.

TABLE 11. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON WIRE STEM OF CABBAGE CAUSED BY CORTICIUM VAGUM B. & C.

Number of trial	Number of tank	Soil		Percentage of damping-off at indicated hours after inoculation												Condition of plants†			
		Temperature (C.).	Moisture (per cent)*	Hours															
				30	45	54	68	74	90	98	105	112	127	136	152	I	II	III	IV
				Per cent of damping-off															
VIII	1	10.5°–13° (Mode 12°)	18	0	0	0	0	0	0	0	0	5	5	5	5	95	5	0	0
			26	0	0	0	0	0	8	15	20	35	73	75	83	0	15	83	2
			34	0	0	3	8	8	8	18	58	68	83	93	93	8	2	85	5
			42	0	0	3	3	5	15	45	65	80	83	85	85	10	10	80	0
			51	0	0	0	0	0	0	5	35	48	72	83	90	3	5	92	0
	2	19°–20.5° (Mode 20°)	18	0	3	3	5	10	20	23	30	33	40	40	40	45	10	35	10
			26	0	0	0	5	20	48	58	78	85	88	88	88	8	5	37	50
			34	0	3	8	53	75	98	98	100	100	100	100	100	0	0	37	63
			42	0	0	3	50	83	93	93	93	93	93	97	97	3	3	47	47
			51	0	3	5	40	73	83	83	87	87	87	87	87	7	16	70	7
	3	22°–23.5° (Mode 23°)	18	0	0	28	70	83	100	100	100	100	100	100	100	0	0	3	97
			26	0	0	15	55	68	88	88	88	88	93	93	93	7	3	25	65
			34	0	3	20	85	88	93	95	95	95	95	95	95	3	5	33	59
			42	0	0	13	58	75	95	98	98	98	98	98	98	2	3	55	40
			51	0	3	45	75	83	95	95	95	95	95	95	95	5	7	73	15

* Percentage of maximum retentive power of soil as determined by the Hilgard method.

† I, healthy; II, slightly diseased; III, badly diseased; IV, dead.

TABLE 12. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON WIRE STEM OF CABBAGE CAUSED BY CORTICIUM VAGUM B. & C.

Number of trial	Number of tank	Soil		Percentage of damping-off at indicated hours after inoculation												Condition of plants†			
		Temperature (C.)	Moisture (per cent)*	Hours															
				30	48	54	68	74	90	98	105	112	127	136	152	I	II	III	IV
				Per cent of damping-off															
IX	1	10°–13°	18	....	....	....	....	Permanent wilting					....	....	....	....	....	....	....
			26	0	0	0	0	0	0	0	2	7	7	14	14	32	20	48	0
		(Mode 12°)	34	0	0	0	0	0	0	8	20	41	54	76	76	0	5	95	0
			42	0	0	0	0	0	0	5	15	38	53	83	83	0	0	100	0
			51	0	0	0	0	0	0	3	15	33	53	83	83	0	0	100	0
	2	18°–19°	18	....	....	....	....	Permanent wilting					....	....	....	....	....	....	....
			26	0	0	0	0	3	13	18	26	51	77	85	85	0	5	87	8
		(Mode 18°)	34	0	0	0	0	41	51	88	95	100	100	100	100	0	0	56	44
			42	0	0	0	0	47	77	97	100	100	100	100	100	0	0	83	17
			51	0	0	5	5	40	58	83	88	100	100	100	100	0	0	52	48
	3	28°–30°	18	....	....	....	....	Permanent wilting					....	....	....	....	....	....	....
			26	0	0	0	0	3	13	20	30	35	40	45	45	42	25	18	15
		(Mode 28°)	34	0	0	12	12	48	69	86	88	90	90	93	93	5	0	15	80
			42	0	0	23	23	75	90	95	98	98	98	98	98	0	5	8	87
			51	0	0	62	26	71	82	93	95	95	95	95	95	0	5	15	80

* Percentage of maximum retentive power of soil as determined by the Hilgard method.

† I, healthy; II, slightly diseased; III, badly diseased; IV, dead.

TABLE 13. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON WIRE STEM OF CABBAGE CAUSED BY CORTICIUM VAGUM B. & C.

Number of trial	Number of tank	Soil		Percentage of damping-off at indicated hours after inoculation															Condition of plants†			
		Temperature (C.)	Moisture (per cent)*	Hours																		
				30	48	72	80	96	104	120	128	144	152	168	172	192	216	240	I	II	III	IV
				Per cent of damping-off																		
X	1	9°–11° (Mode 10°)	12	..	..	..	..	..	....	....	Permanent wilting				....	....	....	....	....	....	....	....
			18	..	..	..	..	..	....	....	Permanent wilting				....	....	....	....	....	....	....	....
			26	0	0	0	0	0	0	0	0	3	3	3	3	6	9	18	38	32	30	0
			34	0	0	0	0	0	0	0	0	6	9	9	18	42	45	58	21	9	64	6
			42	0	0	0	0	0	0	0	0	0	3	3	23	45	52	55	21	9	70	0
	2	13°–15° (Mode 14°)	12	..	..	..	..	..	....	....	Permanent wilting				....	....	....	....	....	....	....	....
			18	..	..	..	..	..	....	....	Permanent wilting				....	....	....	....	....	....	....	....
			26	0	0	0	0	0	0	0	0	0	0	5	5	11	14	14	83	7	10	0
			34	0	0	0	0	0	5	27	44	54	59	64	68	76	78	80	15	0	77	8
			42	0	0	0	0	7	19	65	79	93	95	98	100	100	100	100	0	0	95	5
	3	29°–32° (Mode 31°)	12	..	..	..	..	..	....	....	Permanent wilting				....	....	....	....	....	....	....	....
			18	..	..	..	..	..	....	....	Permanent wilting				....	....	....	....	....	....	....	....
			26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98	2	0	0
			34	0	0	0	0	3	3	3	3	3	5	5	5	5	5	5	95	3	2	0
			42	0	3	10	12	24	27	29	29	29	29	29	29	29	29	38	60	25	2	13

* Percentage of maximum retentive power of soil as determined by the Hilgard method.
† I, healthy; II, slightly diseased; III, badly diseased; IV, dead.

TABLE 14. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON WIRE STEM OF CABBAGE CAUSED BY CORTICIUM VAGUM B. & C.

Number of trial	Number of tank	Soil		Percentage of damping-off at indicated hours after inoculation														Condition of plants†			
		Temperature (C.)	Moisture (per cent)*	Hours																	
				30	48	64	72	90	96	115	123	138	146	160	190	240	264	I	II	III	IV
				Per cent of damping-off																	
XI	1	5.5°–7° (Mode 5.5°)	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
			26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
			34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
			42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
			51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
	2	24.5°–26° (Mode 25°)	18	..	..	..	..	..	..	Permanent wilting				....	....	....	....	....	....	....	....
			26	0	0	0	2	2	4	13	13	18	24	30	30	33	35	61	30	9	0
			34	0	9	33	48	61	66	72	74	76	78	78	80	80	80	15	26	49	10
			42	0	4	42	50	81	83	85	85	85	88	88	88	88	95	13	20	59	8
			51	0	2	14	17	26	26	28	28	28	28	28	28	33	33	73	16	9	2

* Percentage of maximum retentive power of soil as determined by the Hilgard method.

† I, healthy; II, slightly diseased; III, badly diseased; IV, dead.

TABLE 15. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON WIRE STEM OF CABBAGE CAUSED BY CORTICIUM VAGUM B. & C.

Number of trial	Number of tank	Soil		Percentage of damping-off at indicated hours after inoculation															Condition of plants†			
		Temperature (C.)	Moisture (per cent)*	Hours																		
				30	48	72	80	96	104	120	128	144	152	168	172	192	216	240	I	II	III	IV
				Per cent of damping-off																		
XII			18	..	..	..	..	..	....	....	Permanent wilting				....	....	....	....	....	....	....	....
		24°-26°	26	0	0	0	3	3	3	3	3	3	3	4	7	7	7	7	93	7	0	0
	1		34	0	0	2	6	6	6	6	6	6	6	8	14	14	14	14	92	0	8	0
		(Mode 25.5°)	42	0	0	9	14	21	32	36	43	45	46	50	50	50	50	50	25	25	46	4
			51	0	0	12	26	33	40	43	46	50	55	60	60	60	60	60	5	48	47	0
			18	..	..	..	..	..	....	....	Permanent wilting				....	....	....	....	....	....	....	....
		32°-34°	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
	2		34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
		(Mode 32.5°)	42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
			51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0

* Percentage of maximum retentive power of soil as determined by the Hilgard method.

† I, healthy; II, slightly diseased; III, badly diseased; IV, dead.

4. Temperature conditions are a greater factor than the total number of diseased plants, in affecting the rate of development of the disease, since frequently, at the ordinary temperatures, it is but a matter of time until a large proportion of the plants are diseased (tables 9 to 15).

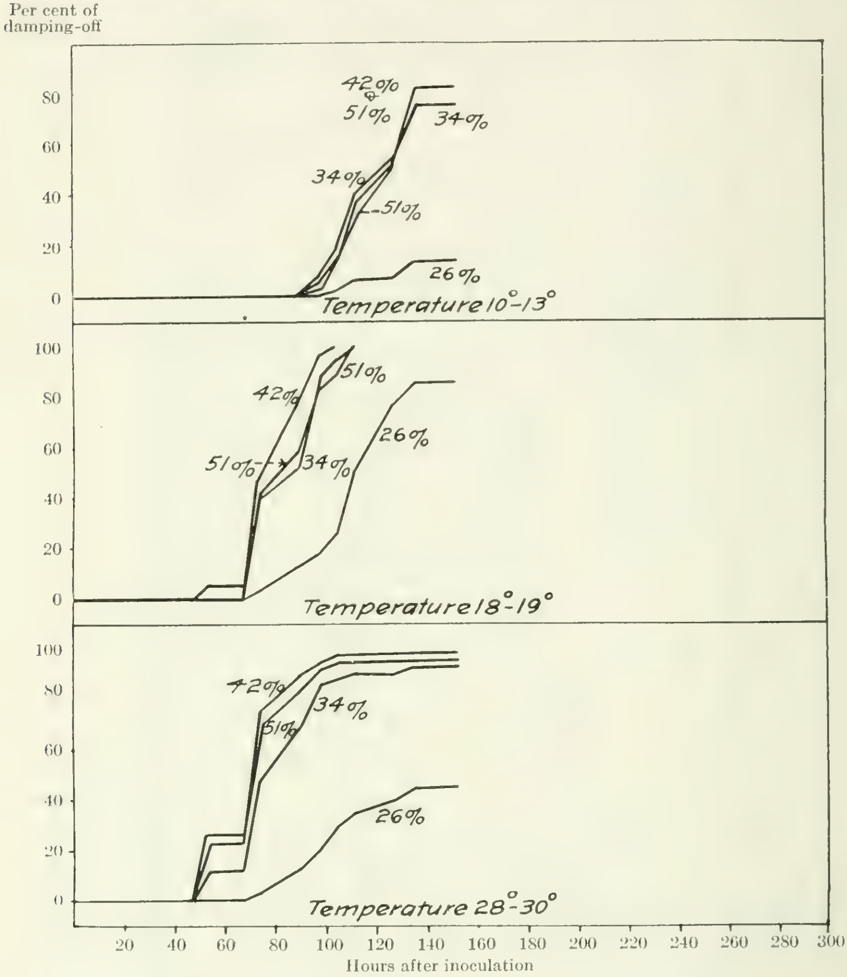

FIGURE 10. INFLUENCE OF DIFFERENT SOIL TEMPERATURES AND SOIL MOISTURES ON RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS CAUSED BY CORTICIUM VAGUM (TABLE 12, TRIAL IX)

Soil moistures are given as percentages

5. Any soil moisture favorable for the growth of the host is favorable for the growth of the fungus, as damping-off resulted at the lowest moistures in which the plants would grow. In a few cases, incipient wilting was observed in the checks of the lower moistures, where the percentage of

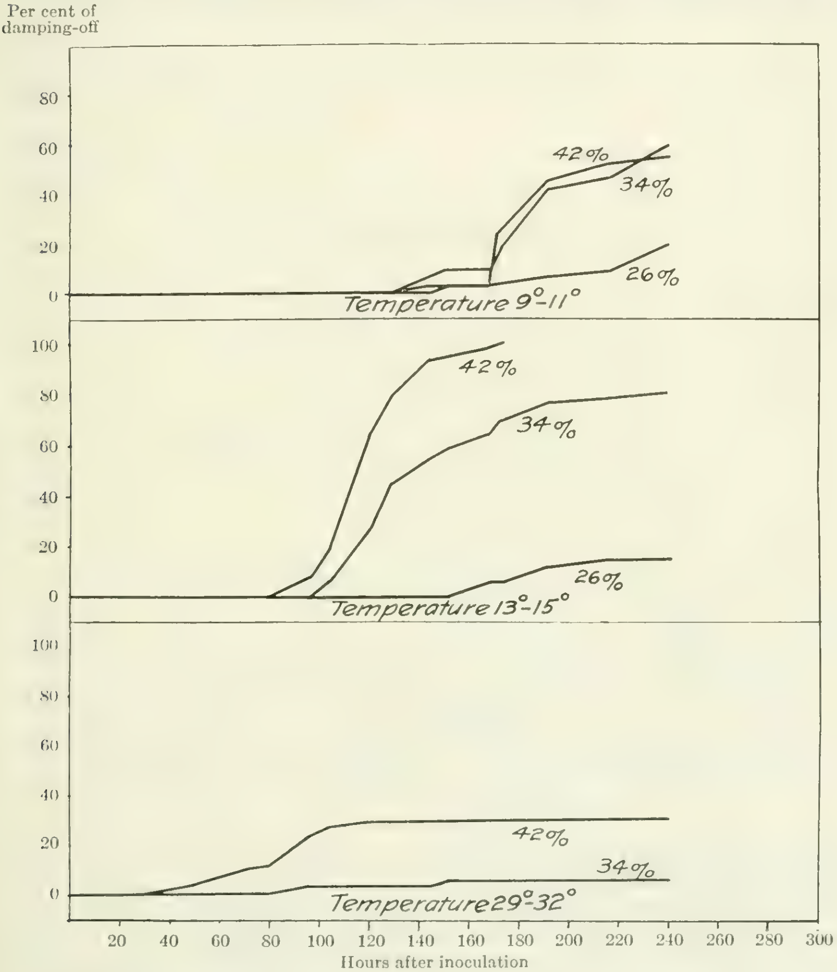

FIGURE 11. INFLUENCE OF DIFFERENT SOIL TEMPERATURES AND SOIL MOISTURES ON RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS CAUSED BY CORTICIUM VAGUM (TABLE 13, TRIAL X)

Soil moistures are given as percentages

damping-off was high. Excess moistures injurious to the parasite were at the same time injurious to the host, but this occurred only in practically saturated soils (tables 10 to 15).

6. A combination of low temperatures and low moistures greatly delays the rate of damping-off. In some instances the total percentage of diseased plants is also decreased, but not materially so in all these instances (trials VI to XII, tables 9 to 15).

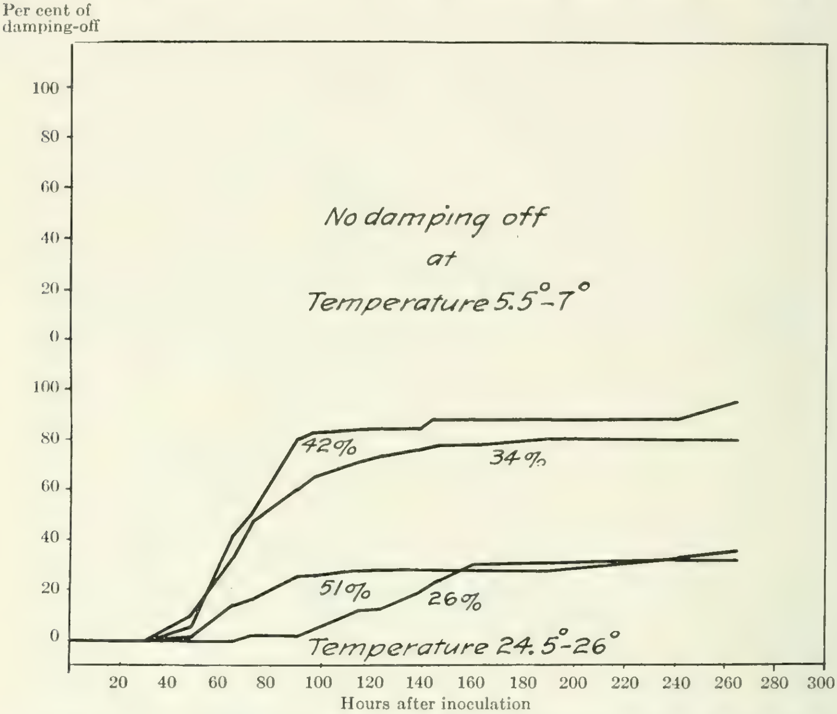

FIGURE 12. INFLUENCE OF DIFFERENT SOIL TEMPERATURES AND SOIL MOISTURES ON RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS CAUSED BY CORTICIUM VAGUM (TABLE 14, TRIAL XI)

Soil moistures are given as percentages

It was hardly to be supposed that plants growing at a very low temperature would in any way acquire a resistance to further attack of the parasite. The fact that they would not was definitely indicated when those plants kept at 5.5° C. (table 14) for 264 hours after inoculation practically all damped-off within 48 hours after being subjected to a temperature of 25° C. Only half of the plants were placed at this temperature at first, and the remainder were kept as controls; but as soon as the first lot had succumbed with such rapidity, the others were likewise placed at that temperature with similar results. This experiment was later duplicated by growing one hundred and eighty plants (twelve culture vessels) at a temperature of 12° C. for three weeks, and another equal number of seedlings at 30° C. for the same length of time, and finally placing them in the same tank at 20° C. They were immediately inoculated by placing the inoculum in direct contact with the seedling. The rate as well as the total percentage of damping-off was practically the same in both sets of seedlings. The moistures were the same in all the culture vessels.

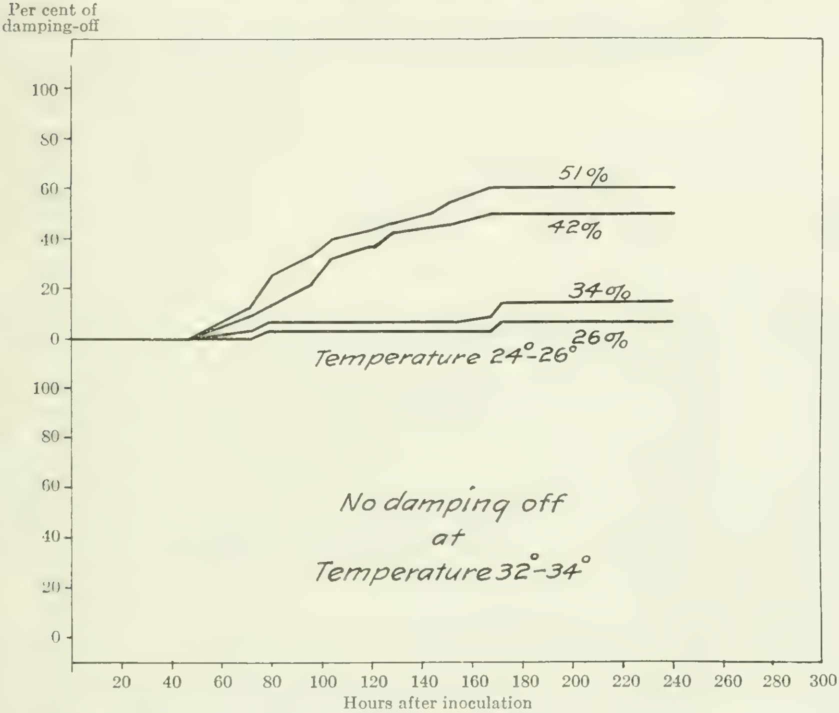

FIGURE 13. INFLUENCE OF DIFFERENT SOIL TEMPERATURES AND SOIL MOISTURES ON RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS CAUSED BY CORTICIUM VAGUM (TABLE 15, TRIAL XII)

Soil moistures are given as percentages

One of the interesting observations in the experiments is that of the definite lag in the rate of damping-off in those trials (VI, XI, and XII, tables 9, 14, and 15) which were performed in April, when the air temperatures were comparatively higher than when the other trials were made. Here is shown the intimate relationship between the air and soil temperatures. Careful air-temperature records were kept at all times. Such records are given in table 16. These temperatures were not so high as the maximum for the growth of the fungus, but were nevertheless much beyond the optimum temperature. They were a deciding factor both directly in the growth of the fungus, and in drying out the surface layer of the soil, thus delaying growth. There is no need of giving further data, but similar figures could be given where a similar increase in the air temperature the previous year was accompanied by a corresponding decrease in the rate of damping-off. The supposition that such decreases were due to the inoculum can hardly hold, as the inoculum for all of these later trials was the same as that used previously and had been prepared a longer time before inoculation than in the earlier experiments. This might have insured even a more thorough infection of the wheat grains with the mycelium, and more sclerotial formation, if that were possible.

TABLE 16. THE DIFFERENT MAXIMUM, MINIMUM, AND AVERAGE AIR TEMPERATURES UNDER WHICH TRIALS VI, XI, AND XII WERE PERFORMED

Number of trial	Temperatures (centigrade)		
	Minimum	Maximum	Average
VI	5°–17°	15°–29°	11°–20°
XI	9°–16°	17°–30°	11°–23°
XII	13°–20°	22°–33°	16°–27°

Bottom as compared with top watering.

The foregoing experiments emphasize the importance of the water content of the surface soil as a factor in determining the health of plants growing in infected soil. This is especially illustrated in trial VIII (table 11), where the plants even in a dry soil damped-off forthwith, because the moisture content in the immediate vicinity of the seedlings had been increased several days previous to inoculation. This is in accordance with the recommendations of Jones (1908) and of Johnson (1914), who suggest keeping the surface soil dry in order to delay damping-off. To obtain some definite data, three culture vessels with a total of thirty-three seedlings, watered by sprinkling the surface, were inoculated, and were compared with two vessels containing twenty-one plants which were given the same amount of water added to the bottom of the soil. All other conditions were similar. There was considerably less damping-off in the latter case, as well as a decrease in the rate (table 17). This experiment was repeated with sixty plants (four culture vessels) for each of

TABLE 17. RESULTS OF PRELIMINARY EXPERIMENT SHOWING DIFFERENT RATE OF DAMPING-OFF IN WET AND DRY SURFACE SOILS

Days after inoculation	Rate of damping-off (per cent)	
	Surface watered	Bottom watered
3	9	0
4	21	0
5	73	14
6	88	24
7	97	48
9	100	52
10	100	62

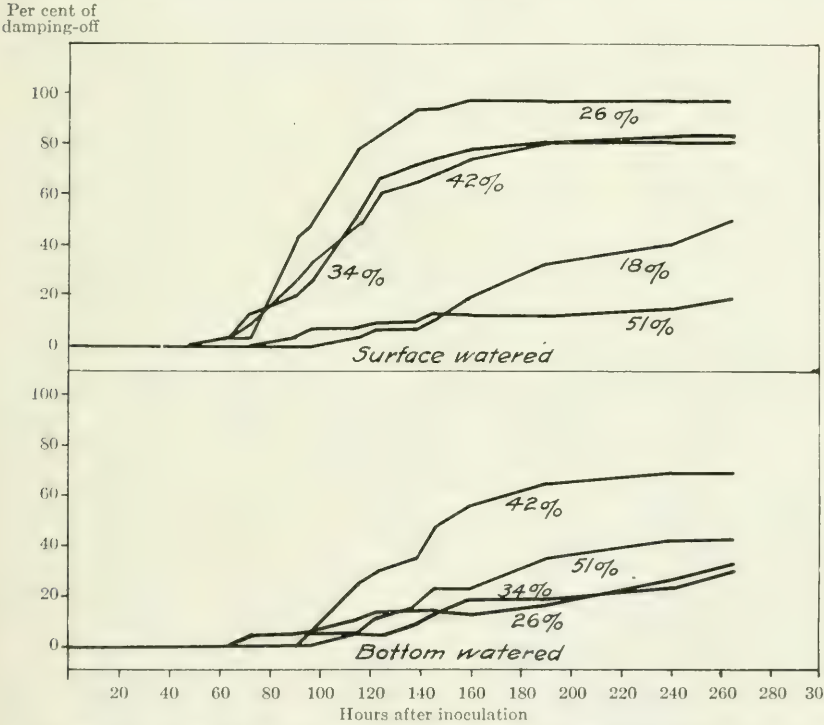

FIGURE 14. DIFFERENCE IN RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS IN SOIL THAT IS SURFACE-WATERED, AND SOIL THAT IS BOTTOM-WATERED (TABLE 18, TRIAL XIII)

Soil moistures are given as percentages

TABLE 18. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON WIRE STEM OF CABBAGE CAUSED BY CORTICIUM VAGUM B. & C.

Number of trial	Number of tank	Soil		Percentage of damping-off at indicated hours after inoculation														Condition of plants†			
		Temperature (C.)	Moisture (per cent)*	Hours														I	II	III	IV
				30	48	64	72	90	96	115	123	138	146	160	190	240	264				
				Per cent of damping-off																	
				Surface watered																	
XIII	3	12°–16°	18	0	0	0	0	0	0	3	7	7	10	20	33	40	50	3	97	0	0
			26	0	0	3	3	44	48	79	85	94	94	97	97	97	97	3	0	72	25
			34	0	0	3	11	19	25	53	67	72	75	78	81	81	81	6	17	77	0
			42	0	0	3	9	26	34	48	60	65	69	74	80	83	83	12	0	88	0
			51	0	0	0	0	3	6	6	8	8	11	11	11	14	19	85	5	5	5
				Bottom watered																	
			18	...	...	...	...	...	...	Permanent wilting				....	....	....	....	96	4	0	0
			26	0	0	0	4	4	4	4	4	8	13	13	16	25	33	42	16	42	0
			34	0	0	0	0	0	5	10	14	14	14	19	19	24	29	55	15	30	0
			42	0	0	0	0	0	4	26	30	35	48	57	65	69	69	26	17	57	0
			51	0	0	0	0	0	0	4	12	15	23	23	35	42	42	58	23	8	11

* Percentage of maximum retentive power of soil as determined by the Hilgard method.

† I, healthy; II, slightly diseased; III, badly diseased; IV, dead.

five moistures, not counting the control plants. Results similar to those in the previous experiment were obtained. Permanent wilting resulted in bottom-watered soil with a moisture content of 18 per cent. Incipient wilting only resulted at that moisture when the water was added to the surface of the soil. The rate as well as the total number of diseased plants was greatly increased by surface watering (table 18 and figure 14). This is a bit of experimental evidence, little of which is found in literature in support of the recommendations mentioned above. The fact that little such evidence is found is probably because it concerns a fact apparently too obvious to require experimental proof.

Effect of surface layer of sand

The question as to the most practicable method of maintaining such a dry surface still remains. Probably a current recommendation is a covering of sand. Johnson (1914) and Sherbakoff (1917 b), however, give rather conflicting opinions. Johnson thinks that such a practice is of doubtful value, while Sherbakoff states:

> It is often advantageous to have on the surface of the seed bed a half-inch layer of pure, coarse sand. Damping off fungi grow on the surface or in upper layers of the soil and thrive best when the soil is well supplied with organic matter. This layer of pure sand will hinder the growth of the fungi.

To obtain some data on this phase of the subject, the following experiments were performed:

1. Naturally infected soil was thoroughly mixed and placed in flats. Seed was sown in the ordinary way and covered very lightly with soil, and a $\frac{1}{4}$-inch layer of clean, coarse sand was placed over the surface. Only 50 per cent of the seedlings pushed through the surface. On investigation it was found that all the seed germinated, but half of the seedlings were destroyed before they reached the surface. All the plants which actually started to grow damped-off later. Apparently the sand, instead of permitting rapid surface evaporation, prevented it and formed an ideal environment at the soil-and-sand juncture for damping-off.
2. Another flat was sown to seed where the soil consisted of a mixture of 50 per cent of infected soil and 50 per cent of sand. Although practically 100 per cent germination occurred, only 8 per cent of the seedlings reached the surface of the soil. All of these damped-off later.
3. Seed was sown on top of the infected soil in another flat, and covered with sand only. Here all germinated and grew, but later damped-off.
4. As a control for the above-mentioned experiments, seed was sown in infected soil containing no sand. Twenty-eight per cent of the seedlings emerged, and all damped-off. All the seed in a flat of sterilized soil germinated and remained disease-free.
5. At a later date, seed was again sown in several flats of infected soil and the surface was covered with coarse sand. These flats were all given

the same amount of water. Again the germination was 100 per cent, but only 35 per cent of the plants came through the soil, the remaining 65 per cent exhibiting definite, blackened, dead sprouts that had emerged from the seeds. Of the 35 per cent, all developed wire stem. All the seeds not covered with sand reached the surface of the soil, and only one-third of these damped-off or developed wire stem.

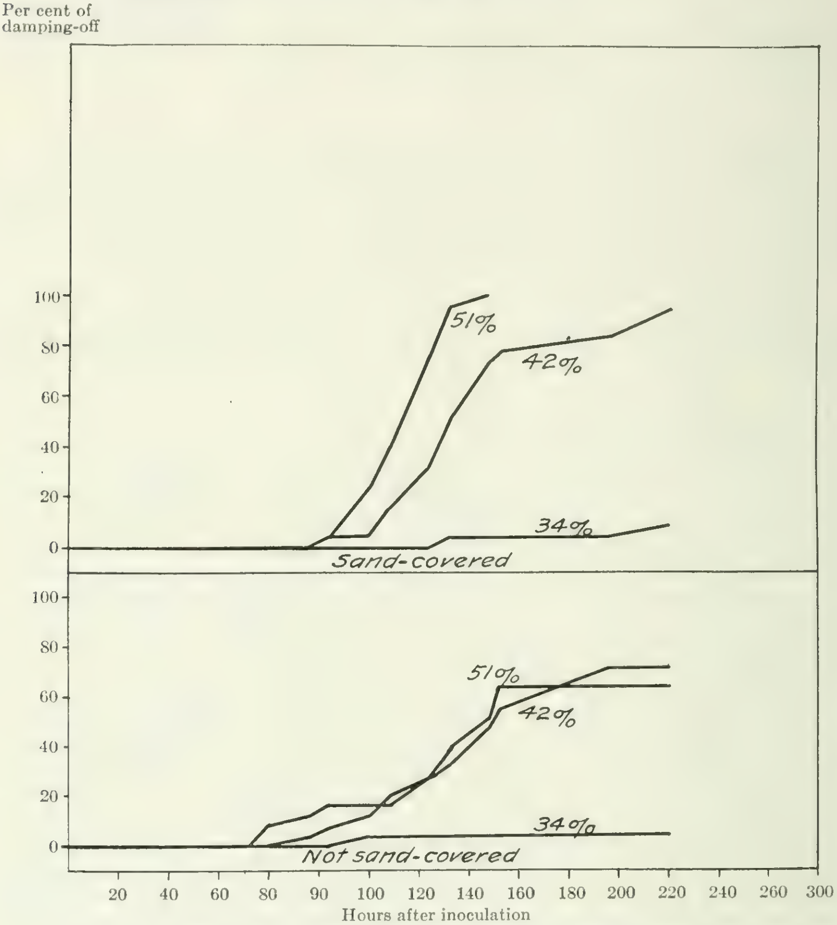

FIGURE 15. INCREASE IN RATE OF DAMPING-OFF OF CABBAGE SEEDLINGS IN SOIL COVERED WITH A LAYER OF FINE QUARTZ SAND (TABLE 19)

Soil moistures are given as percentages

These experiments indicate that sand may even be detrimental. Whether or not this is actually so in every case cannot be stated positively until further experimental work has been done.

Another experiment was performed to determine the influence of a surface layer of sand on the evaporation of water from the soil. Fine quartz sand was used, instead of the coarse sand employed in the previous experiments. Four culture vessels were used in each of five moistures. All the cultures were watered through glass tubes extending to the bottom of the vessels. About fifteen seedlings were transplanted into each jar as before, and were allowed to grow for a week. Inoculations were then made, and immediately the surface of two of the culture vessels in each moisture series was covered with a layer of sand about one-quarter inch deep. The moisture content was kept constant by weighing every other day and adding the required amount of water. During the entire experiment the sand-covered vessels lost only about half as much water as did those that were not covered. It was thus to be expected that the covered vessels were the more ideal for the development of the disease, and later observations demonstrated this to be the case (table 19 and figure 15).

TABLE 19. INFLUENCE OF A LAYER OF QUARTZ SAND ON THE SURFACE OF THE SOIL, ON DAMPING-OFF CAUSED BY CORTICIUM VAGUM B. & C.

Number of tank	Soil moisture* (per cent)	Percentage of damping-off at indicated hours after inoculation. Soil temperature, 16–20° C.											
		Hours											
		72	80	96	104	120	128	144	152	168	172	216	240
		Per cent of damping-off											
		Sand-covered											
3	18	....	....	....	....	Permanent wilting				....	....	....	....
	26	0	0	0	0	0	0	0	0	0	0	0	0
	34	0	0	0	0	0	0	0	4	4	4	4	8
	42	0	0	0	4	4	16	32	52	74	78	84	96
	51	0	0	0	4	24	40	76	96	100	100	100	100
		Not sand-covered											
	18	....	....	....	....	Permanent wilting				....	....	....	....
	26	0	0	0	0	0	0	0	0	0	0	0	0
	34	0	0	0	0	4	4	4	4	4	4	4	4
	42	0	8	12	16	16	16	28	32	48	56	72	72
	51	0	0	4	8	12	20	28	40	52	64	64	64

* Percentage of maximum retentive power of soil as determined by the Hilgard method.

CONTROL

Although the subject of the control of wire stem was not investigated thoroughly, certain phases of the experiments described above are important in indicating lines of attack. The disease cannot be controlled by changes in the environmental conditions. A low temperature and a dry surface soil may reduce the disease or delay it, but not sufficiently to warrant such a procedure as a control measure. The fungus must be killed before the seed or the seedlings are placed in the infected soil, if freedom from the disease is to be insured. The method of eradication is obviously soil sterilization. This can best be done by thorough steaming of the soil or by drenching it with formaldehyde. The latter method was used as recommended by Johnson, with good results (Johnson, 1914, and Sherbakoff, 1917, a and b). Calcium hypochlorite, up to sufficient quantities to kill the host plant, had no value in controlling the disease when used as a soil disinfectant. The Cheshunt mixture (Bewley, 1921–22) was tried at the recommended strength on several occasions. At every trial there was a delay of several days in the appearance of the disease, but later no difference was observed in the percentage of diseased plants growing in treated and in untreated soil.

SUMMARY

Wire stem of cabbage is caused by a strain of *Corticium vagum* B. & C. which is physiologically distinct from the strains causing lesions on potato stems. Of the strains from potatoes with which the writer worked, none were found to be pathogenic to cabbage, and likewise the strain from cabbage seedlings did not produce lesions on potato stems.

The minimum and maximum temperatures for the growth of the fungus in pure culture are approximately 9° and 31° C. The optimum is not sharply defined, but it lies between 22° and 26° C.

The fungus exhibits a wide pH range for growth, the minimum being approximately 2.0 and the maximum above 10.4. The optimum is about 6.2.

Practically any combination of soil temperatures and moistures favorable for the growth of the host is favorable for the growth of the fungus and for the development of wire stem. Consequently, no changes in the environmental conditions will control the disease.

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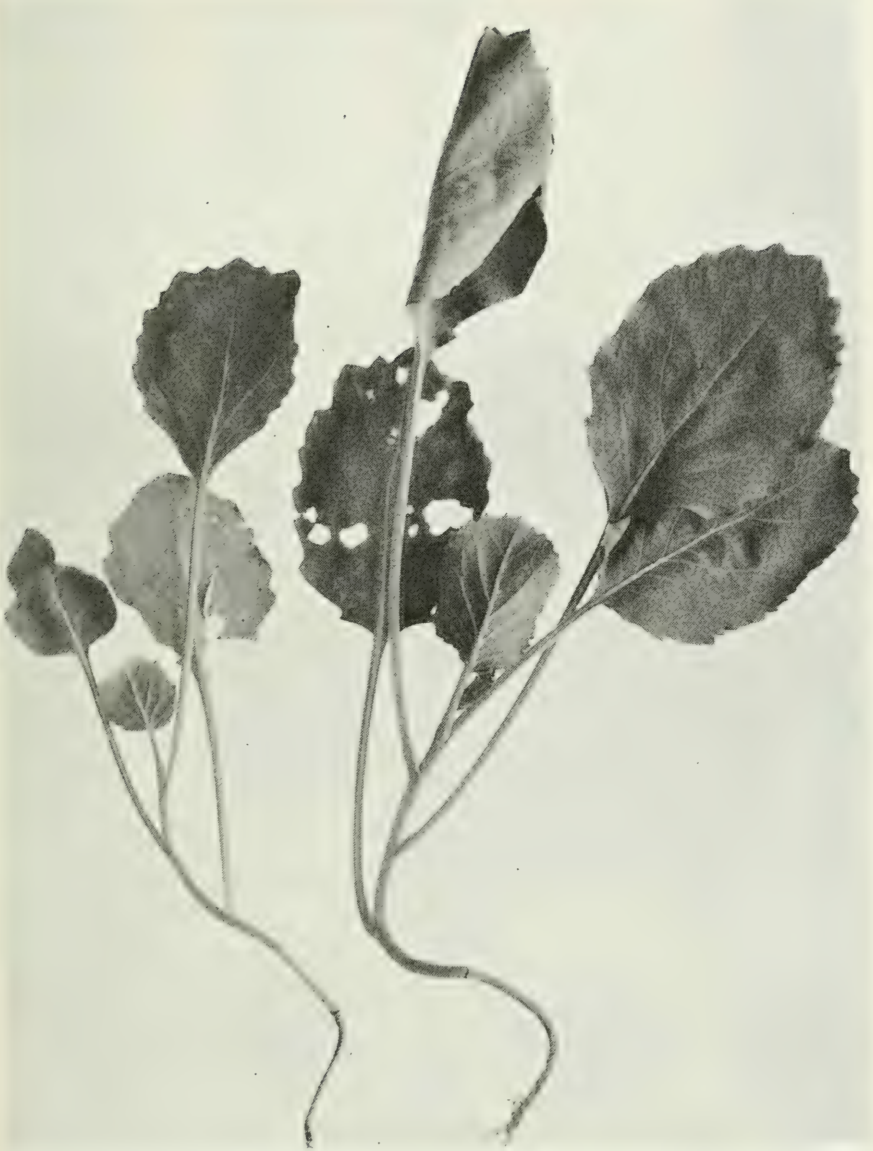
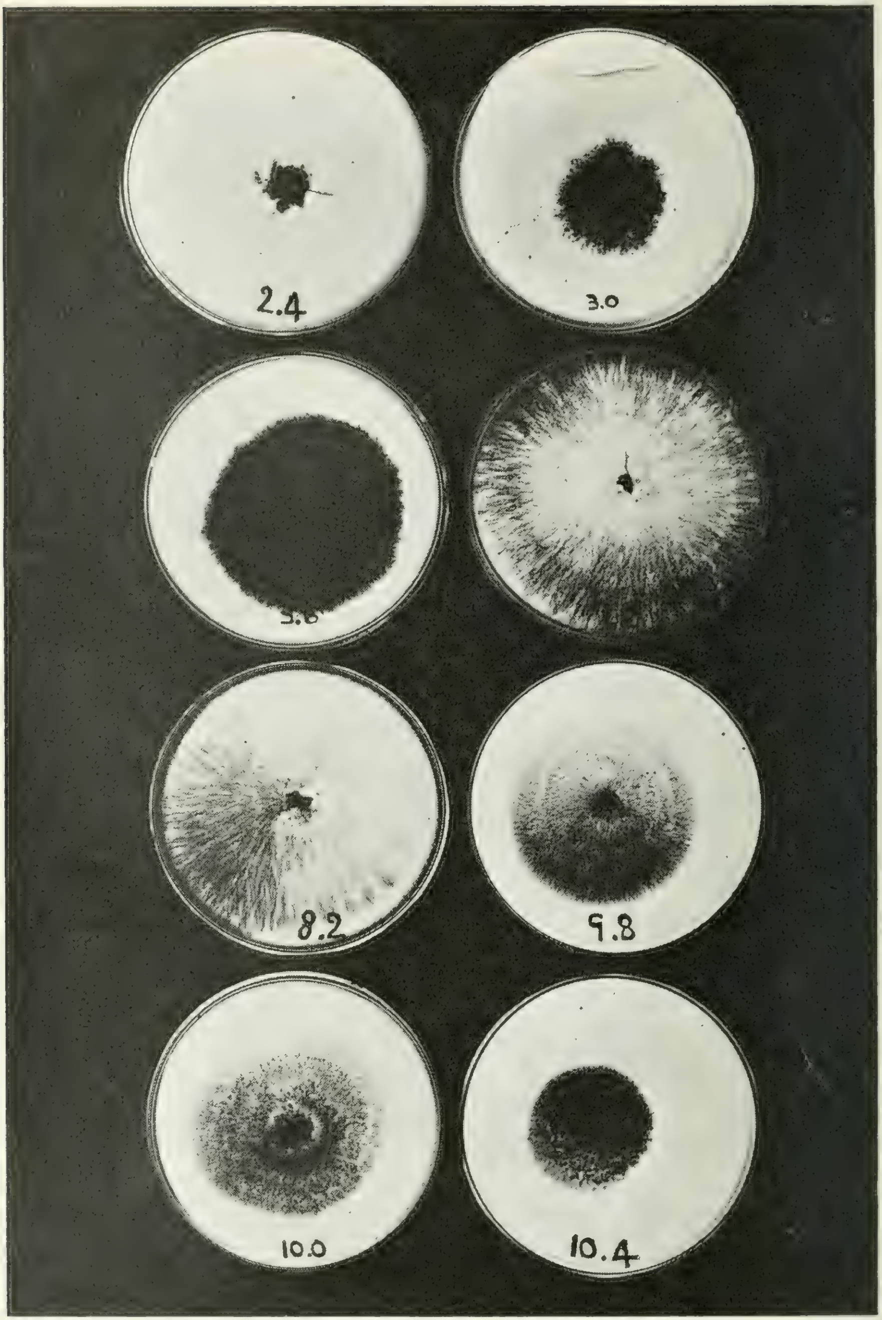
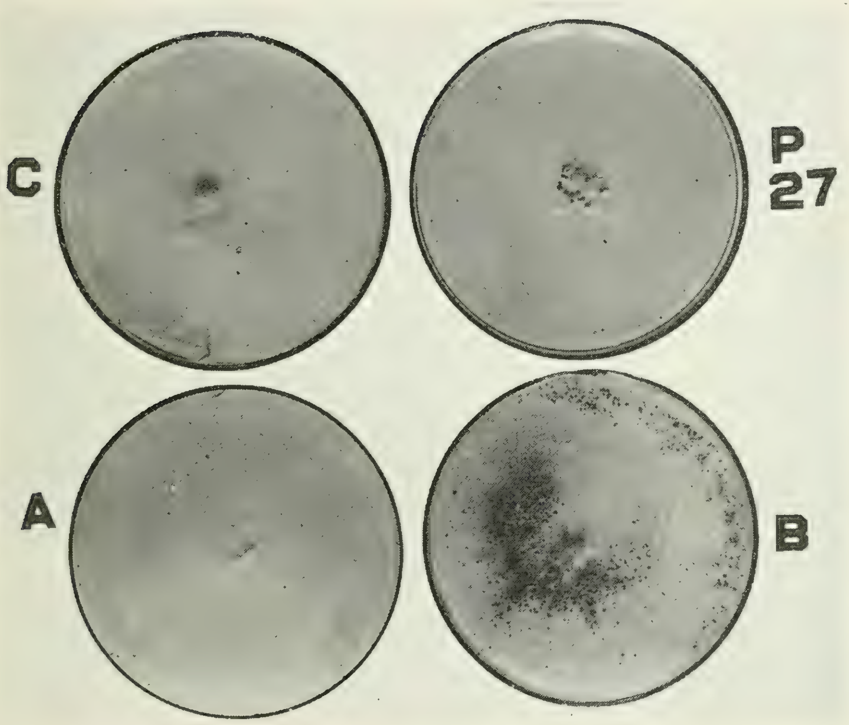


CABBAGE SEEDLINGS AFFECTED WITH WIRE STEM

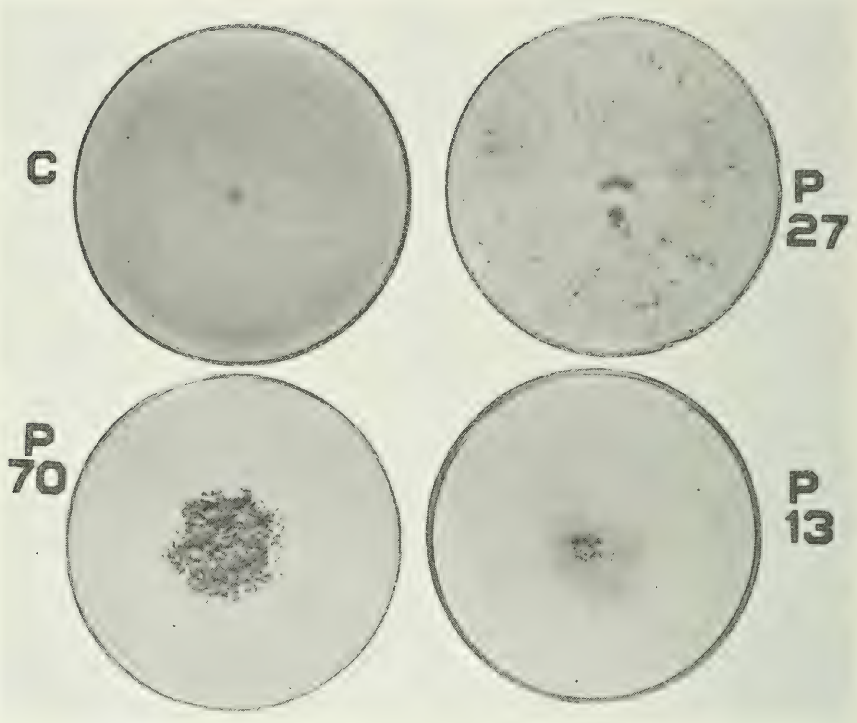


FIVE-DAYS-OLD CULTURES OF CORTICIUM VAGUM ON POTATO AGAR ADJUSTED TO THE pH VALUES AS INDICATED BY FIGURES ON THE PETRI DISHES



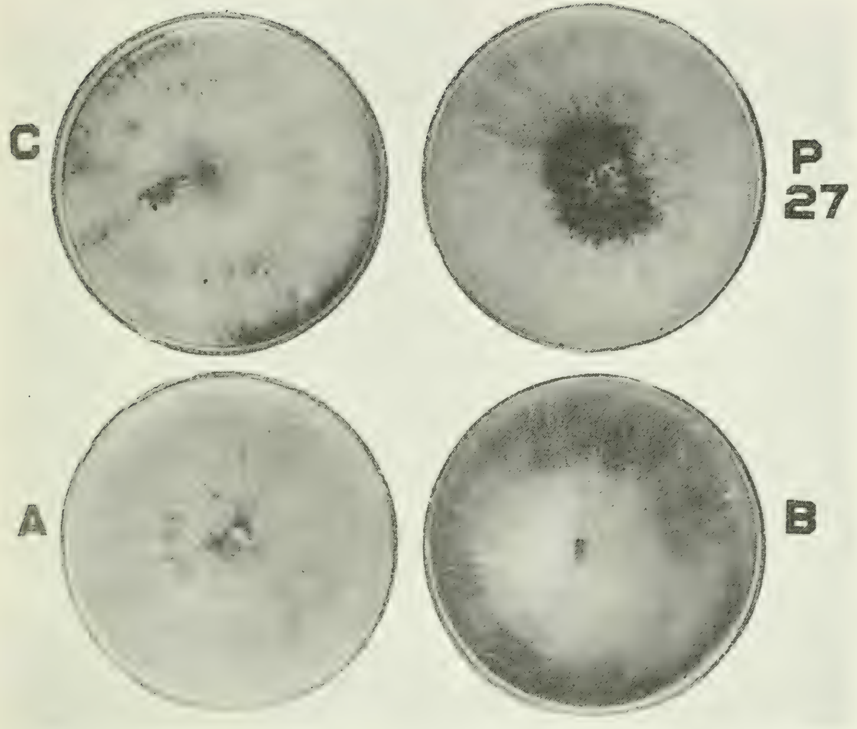
RESPONSE OF FOUR STRAINS OF CORTICIUM VAGUM TO A MEDIUM ADJUSTED TO pH 2.4

C, Cabbage strain, petri dish completely covered with white mycelium. A, Aster strain, petri dish completely covered with white mycelium. P27, Potato strain, showing growth of small diameter, petri dish covered with sclerotia. B, Bamboo strain, petri dish completely covered with a fluffy mass of mycelium, with small sclerotia scattered over surface



RESPONSE OF CABBAGE STRAIN AND OF THREE POTATO STRAINS OF CORTICIUM VAGUM TO A MEDIUM ADJUSTED TO pH 2.7

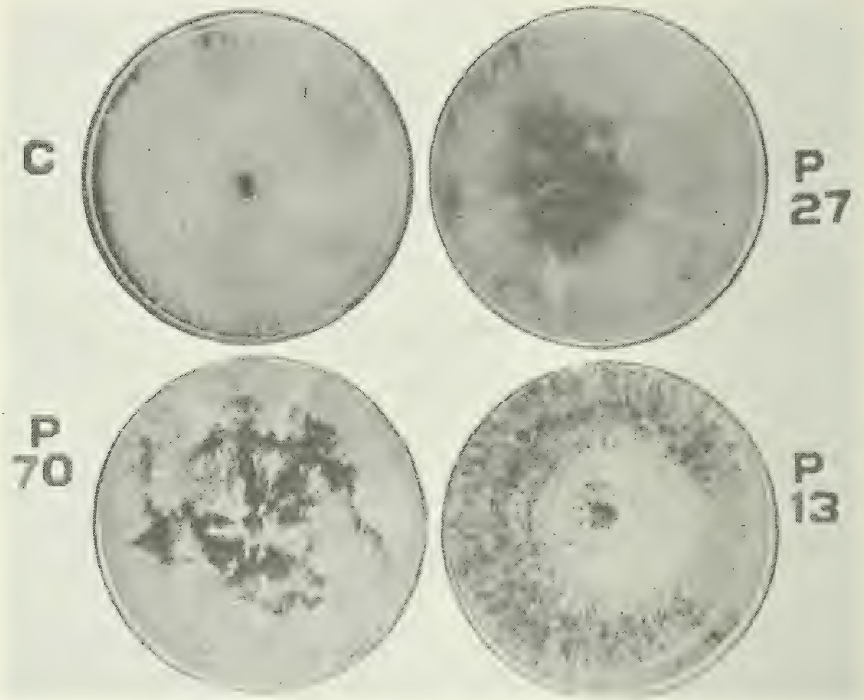
C, Cabbage strain, petri dish completely covered but no sclerotia produced. P27, potato strain, petri dish completely covered, with a few sclerotia scattered on surface. P70, Potato strain, petri dish not covered, except for an area of 2.6 centimeters in diameter which is completely covered with sclerotia. P13, Potato strain, petri dish completely covered, with a few sclerotia in center



DIFFERENT RATES OF FORMATION OF SCLEROTIA OF FOUR STRAINS OF CORTICIUM VAGUM ON POTATO AGAR ADJUSTED TO pH 7.2

(Age of culture, 12 days)

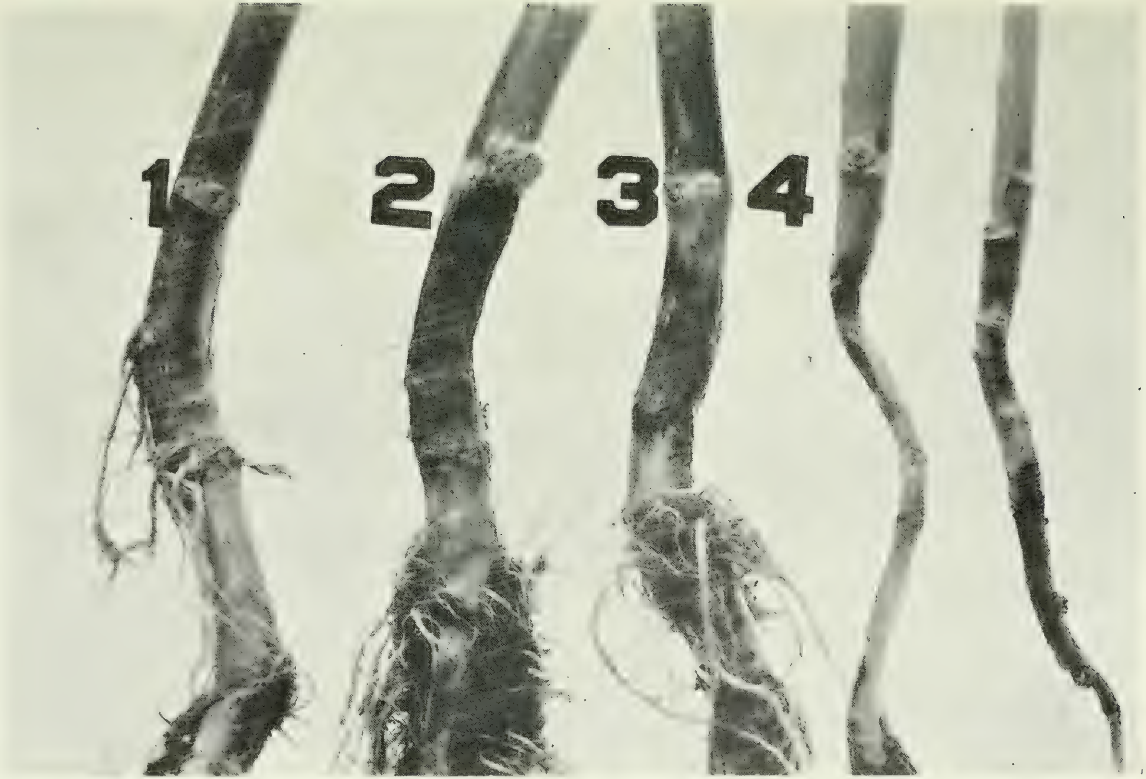
C, Cabbage strain, with but few sclerotia and with brown mycelium at edges of petri dish. A, Aster strain, with but few minute sclerotia. P27, Potato strain, with a heavy mass of sclerotia in center of petri dish. B, Bamboo strain, petri dish practically covered with dark brown mycelium, with a few minute sclerotia



DIFFERENT HABITS OF FORMATION OF SCLEROTIA EXHIBITED BY THE CABBAGE STRAIN AND BY THREE POTATO STRAINS OF CORTICIUM VAGUM

(Age of culture, 12 days)

C, Cabbage strain, with a few minute sclerotia. P27, Potato strain, with large, compact masses of sclerotia in center of petri dish. P70, Potato strain, with uneven, grayish brown sclerotia radiating from center of petri dish. P13, Potato strain, with cinnamon brown sclerotia uniformly scattered over surface of petri dish



CABBAGE STEMS SHOWING SUSCEPTIBILITY OF PLANTS OF DIFFERENT AGES TO CORTICIUM VAGUM

1, Stem not inoculated. 2, Stem inoculated at 18 weeks, showing definite lesions. 3, Stem inoculated at 16 weeks, showing definite lesions. 4, Stem inoculated at 12 weeks, showing definite lesions

MARCH, 1925

MEMOIR 88

CORNELL UNIVERSITY

AGRICULTURAL EXPERIMENT STATION

THE TAKE-ALL DISEASE OF CEREALS AND GRASSES CAUSED BY OPHIOBOLUS CARICETI (BERKELEY AND BROOME) SACCARDO

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THE TAKE-ALL DISEASE OF CEREALS AND GRASSES CAUSED BY OPHIOBOLUS CARICETI (BERKELEY AND BROOME) SACCARDO[1]

ROBERT S. KIRBY

This disease, long known in Europe, Japan, and Australia, was first correctly reported from America in 1920 (Kirby and Thomas, 1920). Reports of the finding of take-all in Illinois appeared as early as 1919 (Humphrey and Johnson, 1919, and Humphrey, Johnson, and McKinney, 1921) but this was a case of mistaken identity, further investigations (McKinney, 1923) having shown that disease to be distinct from take-all. It is now known as the rosette disease of wheat.

Because of the severity of take-all in certain foreign countries, much concern has been expressed lest it appear in the United States and cause heavy losses to cereals. Since the value of wheat, oats, rye, and barley in the State of New York in 1919, according to the fourteenth census, was equal to about that of one-ninth of all crops grown, or nearly equal to the value of the fruit or the potato crop in the State, the discovery of the disease here seemed to warrant a careful study of its distribution and severity under New York State conditions. The present investigations cover a period of four years during which time the disease has been studied in the field and greenhouse.

HOST RANGE

As far as is known, take-all is restricted to the Gramineae. An examination of the data given in table 1 shows that of the two subfamilies of the Gramineae, fewer members of the Panicoideae appear to be subject to the disease than of the Poacoideae. Of these species which were investigated, two out of fourteen in the Panicoideae, and eighty-three out of one hundred and forty-two in the Poacoideae, are susceptible. In the Panicoideae, susceptible species are found in only one of the three tribes investigated; in the Poacoideae all of the seven tribes tested contain forms liable to infection.

The tribe Hordeae seems to contain more susceptible species than any other tribe in the Gramineae. The other tribes, arranged according to the number of susceptible species in them, are as follows: Agrostidae, Festuceae, Phalarideae, Avenae, Oryzeae, Paniceae, and Chlorideae.

In no tribe were all of the species found to be susceptible, but all of the species inoculated in a few of the genera — Agropyron, Elymus,

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[1] Also presented to the Faculty of the Graduate School of Cornell University, June, 1923, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

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Hordeum, Hystrix, Phleum, and Stipa — became affected. Of all the genera tested, the fewest resistant species were found in Agropyron, Elymus, Hordeum, Hystrix, Stipa, and Triticum. Of these, the species in Triticum seem to be the most susceptible since they are the only hosts to show any marked degree of hypoplasia.

RELATIVE SUSCEPTIBILITY OF GRASSES AND OF CEREALS

Grasses

The most important rôle of the grass hosts, excluding the cereals, is as harborers of the pathogene. Since the importance of the individual host in this respect depends upon the number of perithecia produced on it, the evidence presented in table 1 is given in terms of the relative number of perithecia. The number of perithecia was determined for each species by dissecting numerous culms under the binocular. On all grasses reported as hosts, typical perithecia and ascospores have been found.

The host-range tests made in the greenhouse and tabulated in table 1 represent a repetition and extension of those previously reported by the

TABLE 1. HOST RANGE OF *Ophiobolus cariceti* AS OBSERVED FROM INOCULATION TESTS IN THE GREENHOUSE, OBSERVATIONS IN THE FIELD, AND REPORTS OF OTHER INVESTIGATORS

Name of grass *	Relative number of perithecia on plant †		Presence of perithecia previously recorded ‡
	In greenhouse tests	In the field	
Agropyron sp.	....	....	(1)
caninum (L.) Beauv.	Many.....	....	(14)
cristatum J. Gaertn.	Many.....	....	(14)
desertorum (Fisch.) Richt.	Very many	....	....

---

* All the grasses in this test were grown from seed obtained from the following sources. Dr. E. B. Mains, Purdue University, LaFayette, Indiana; Professor A. H. Larson, Seed Laboratory, University of Minnesota, St. Paul, Minnesota; Berkeley, California, grass gardens of the Cereal Office of the United States Department of Agriculture.

† Few=less than ten perithecia per culm.
Moderate=from ten to forty perithecia per culm.
Many=more than forty perithecia per culm.

‡ References to literature in last column:
1=Saccardo (1875)
2=Waters (1920a)
3=Brittlebank (1920)
4=Berkeley and Broome (1861)
5=Anonymous (1919)
6=McAlpine (1904)
7=Rosen and Elliott (1923)
8=Anonymous (1907)
9=Hori (1901)
10=Peglion (1898)
11=Stakman (1922)
12=Pridham (1919)
13=Anonymous (1922a)
14=Kirby (1922)

TABLE 1 (*continued*)

Name of grass *	Relative number of perithecia on plant †		Presence of perithecia previously recorded ‡
	In greenhouse tests	In the field	
Agropyron (*continued*):			
intermedium (Host.) Beauv.	Many		(14)
obtusiusculum Lange	Moderate		
repens (L.) Beauv.	Many	Moderate	(2)
richardsoni Schrad.	Many		
scabrum (R. Bv.) Beauv.			(3)
smithii Rydb.	Moderate		(14)
spicatum (Pursh) Rydb.	Many		
tenerum Vasey	Many		(14)
Agrostis canina L.	None		
palustris Huds.	None	Few	
(=*A. alba* L.)			
perennans (Walt.) Tuckerm.	Few		
retrofacta Willd.	Few		
stolonifera L.	None		
verticillata Vill.	Few		
Aira caespitosa L.			(4)
caryophyllea L.	None		
Alopecurus geniculatus L.	Moderate		
pratensis L.	None		
Andropogon scoparius Michx.	None		
Anthoxanthum odoratum L.	Many		
Arrhenatherum elatius (L.) Beauv.	None		
Avena brevis Roth	None		
fatua L.	None		
nuda L. (hull-less oats)	None		
orientalis Schreb. (side oats)	None		
sativa L. (common oats)	None	None	(5)

---

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† Few=less than ten perithecia per culm.
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14=Kirby (1922)

TABLE 1 (*continued*)

Name of grass *	Relative number of perithecia on plant †		Presence of perithecia previously recorded ‡
	In greenhouse tests	In the field	
Avena (*continued*):			
sterilis L.	None		
strigosa Schreb.	None		
Beckmannia erucaeformis (L.) Host	Many		
Bouteloua curtipendula (Michx.) Torr.	None		
gracilis (HBK.) Lag.	None		
Briza maxima L.	None		
Bromus arvensis L.	Moderate		(14)
brizaeformis Fisch. & Mey.	Moderate		
carinatus Hook. & Arn.	Few		
ciliatus L.	Many		(14)
erectus Huds.	Moderate		(14)
exaltatus Bernh.	Many		
hordeaceus L. (=*B. mollis* L.)	Moderate		(3)
inermis Leyss.	None		
japonicus Thunb.	Few		(14)
lanuginosus Poir.	Many		
madritensis L.	Moderate		(14)
marginatus Nees	Moderate		
pendulinus Sesse	Few		
pumpellianus Scribn.	Moderate		
racemosus L.	Moderate		(14)
rigidus Roth	Moderate		
secalinus L.	Moderate		(14) (7)
sterilis L.	Many		(6)
subvelutinus Shear	Moderate		
tectorum L.	Many		(14)
tectorum nudus Klett & Richter	Many		

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TABLE 1 *(continued)*

Name of grass *	Relative number of perithecia on plant †		Presence of perithecia previously recorded ‡
	In greenhouse tests	In the field	
Bromus *(continued)*:			
unioloides (Willd.) HBK	None		
villosus Forsk	None		
Calamagrostis canadensis (Michx.) Beauv	None		
Capriola dactylon (L.) Kuntze	None		
Cenchrus echinatus L	None		
tribuloides L	None		
Chaetochloa geniculata (Lam.) Millsp. & Chase			(7)
lutescens (Weigel) Stuntz	None		
Chloris virgata Swartz	None		
gayana Kunth	None		
Cynodon sp			(1)
Cynosurus cristatus L	None		
Dactylis glomerata L	None		
Danthonia intermedia Vasey	None		
Deschampsia elongata (Hook.) Munro	None		
Digitaria sanguinalis (L.) Scop	None		
Echinochloa crus-galli (L.) Beauv	None		
frumentacea (Roxb.) Link	None		
Elymus arenarius L	Many		
australis Scribn. & Ball	Many		(14)
canadensis L	Many		(14)
condensatus Presl	Many		
curvatus Piper	Many		
europaeus L	Many		
giganteus Vahl	Few		
hirsutiglumis Scribn. & Sm	Few		
sibiricus L	Moderate		

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TABLE 1 (*continued*)

Name of grass *	Relative number of perithecia on plant †		Presence of perithecia previously recorded ‡
	In greenhouse tests	In the field	
Elymus (*continued*):			
virginicus L.	Many		(14)
Eragrostis lugens Nees	None		
Festuca bromoides L.	None		
elatior L.	Few		(14) (7)
heterophylla (Lam.) Hack.	None		
megalura Nutt.	None		
octoflora Walt.			(7)
ovina L.	None		
ovina tenuifolia (Sibth.) Sm.	None		
pratensis Huds.	None		
rubra L.	None		
Gastridium lendigerum (L.) Gaud.	Few		
Glyceria grandis Wats.	None		
nervata (Willd.) Trin.	None		
Holcus lanatus L.	Few		
Hordeum distichon L.	Moderate		
jubatum L.	Many		(14)
murinum L.	Few		(8)
nodosum L.	Many		
pusillum Nutt.	None		(7)
secalinum Schreb.	Many		
spontaneum C. Koch	Many		
vulgare L. (barley)	Many	None	(9)
Hystrix patula Moench	Many		(14)
Koeleria cristata (L.) Pers.	Many		
Lolium multiflorum Lam.	None		
perenne L.	None		

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TABLE 1 (*continued*)

Name of grass *	Relative number of perithecia on plant †		Presence of perithecia previously recorded ‡
	In greenhouse tests	In the field	
Lolium (*continued*):			
subulatum Vis	None		
temulentum L	Few		(14)
Oryza sativa L	None		(9)
Oryzopsis miliacea Benth. & Hook	Many		
Panicum virgatum L	Few		
Paspalum setaceum Michx	None		
Pennisetum villosum R. Br	None		
Phalaris arundinacea L	Few		(14)
bulbosa L	Few		
caroliniana Walt	None		
minor Retz	None		
Phleum pratense L	Few	Few	
Phragmites communis Trin	None		
Poa alpina L	Few		
annua L	None		
caesia Sm	None		
chaixii Vill	Few		
compressa L	Moderate	Moderate	
palustris L	Moderate		
pratensis L	Few		
Polypogon littoralis Sm. (With.)	None		
maritimus Willd	None		
monspeliensis (L.) Desf	Moderate		
Poppophorum vaginatum Buckl	None		
Puccinellia simplex Scribn	None		
Secale cereale L. (rye)	Few	Few	(10)
Sesleria coerulea (L.) Scop	None		

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TABLE 1 (*concluded*)

Name of grass *	Relative number of perithecia on plant †		Presence of perithecia previously recorded ‡
	In greenhouse tests	In the field	
Sorghum sudanense (Piper) Stapf	None	....	....
Sporobolus interruptus Vasey	None	....	....
Stipa capillata L.	Moderate	....	....
lepida Hitchc.	Moderate	....	....
pennata L.	Few	....	....
spartea Trin.	Moderate	....	....
viridula Trin.	Few	....	....
Triodia flava (L.) Hitchc.	None	....	....
Triticum aegilopoides Bal. (wild small spelt)	Many	....	....
compactum Host (club wheats)	Many	None	(11)
dicoccoides Körn. (wild emmer)	Heavy	....	(14)
dicoccum Schr. (emmer)	Many	Many	(14)
durum Desf. (durum wheats)	Many	None	(13)
monococcum L. Einkorn	Few	None	(14)
polonicum L. (Polish wheat)	Moderate	....	(14)
spelta L. (large spelt)	Moderate	Moderate	(14)
turgidum L. (poulard wheats)	Many	None	(14)
vulgare Vill. (common wheats)	Many	Many	(12)
Zea mays L. (field corn)	None	....	....

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writer (Kirby, 1922). The method used was essentially the same, except that sterilized soil was used throughout and the inoculum consisted of many more pure-culture isolations. These tests were started on January 3 and 4, 1922, and continued for a period of one year.

Six species of grasses — *Anthoxanthum odoratum*, *Bromus hordeaceus*, *Bromus villosus*, *Holcus lanatus*, *Poa compressa*, and *Poa pratensis* — which were reported as immune in the first tests, were found during the second tests to be susceptible to the take-all pathogene. All grasses found to be hosts in the first series of tests became affected again in the second.

The grasses reported as hosts under field conditions were, in every case, found in wheat fields infested with take-all. Infected *Agropyron repens* was observed in nearly all of the 137 take-all-infested wheat fields visited in 1922 and 1923 and this seems to be the most susceptible wild grass in New York. *Poa compressa* was found infected in Tompkins County only, where it was almost as heavily infected as *Agropyron repens*. *Bromus secalinus*, *Agrostis palustris*, and *Phleum pratense* were found to be infected in Cayuga, Wayne, and Tompkins Counties, but never more than one or two perithecia could be found on a plant. Under field conditions in New York State, however, the grasses appear to be tolerant to the take-all organism, since they are rarely severely stunted even when heavily infected.

Cereals

Apparently take-all is restricted to the small grains. Concerning their relative susceptibility, Hori (1901), reporting on the situation in Japan, states that barley and rye are slightly more susceptible than wheat since they exhibit a greater degree of stunting and have their yields reduced more than does wheat. Oats are reported in Australia (Anonymous, 1919) as being less affected than wheat and barley, but not immune, while Richardson (1911) reports that oats are not affected by take-all. Rice has been reported by Hori (1901) and Tanaka (1917) as being very slightly susceptible to take-all.

The present investigations indicate that spring-planted barley and oats growing under field conditions either escape or are immune to take-all, and that spring-planted wheat becomes infected only under conditions which are extremely favorable to the pathogene. Rye is apparently very much more resistant than wheat. When winter rye and ten varieties of winter wheat were grown in a field test in 1922 to determine their relative susceptibility, 87 per cent of the rye plants and 96.6 per cent of the wheat plants were diseased. Among these diseased plants there was no stunting of the rye plants, but 75.6 per cent of the wheat plants were stunted. This difference in resistance is further borne out by the fact that in badly infested wheat fields volunteer rye is very seldom diseased.

Under greenhouse conditions wheat was the most susceptible of the cereals; barley was more resistant that wheat but less resistant than rye, which was very slightly affected; oats and rice appeared to be immune.

It seems that under New York conditions winter wheat is the only cereal which will be seriously damaged by take-all. However, in warmer sections of the United States where winter barley is grown, the disease may cause a considerable loss to that crop. Rye will probably never suffer any appreciable injury from the disease in New York. Spring-planted cereals, apparently because of their shorter growing season, usually escape the disease, and therefore it is unlikely that they will ever suffer an appreciable loss from it.

VARIETAL SUSCEPTIBILITY IN WHEATS

No variety of wheat has been found which is immune to take-all, although there seem to be marked varietal differences in susceptibility. Pridham (1919) found on testing eighty-one varieties that the percentage of badly infected plants varied from 1 to 33.7 per cent. Previous workers agree that red wheats are more resistant than white wheats. In the Union of South Africa (Anonymous, 1922 a), take-all is said to attack the hard durum types more than the soft *vulgare* wheats. Pridham (1919) reports early-maturing wheats to be the most nearly free from the disease in Australia, while in England (Anonymous, 1913) and in New Zealand (Waters, 1920 a) early or short-season varieties are held to be the most susceptible. Spring wheats are reported as escaping the disease (Foex, 1919 b).

In a previous paper (Kirby, 1922) the writer reported tests with sixty-two varieties of wheat for relative susceptibility under greenhouse conditions. These tests showed that common, or *vulgare*, wheats exhibit about the same degree of susceptibility whether they are of spring or winter habit, soft or hard, and red or white. Of the various kinds of wheat the durum and poulard wheats were all heavily infected, and these are probably the most susceptible varieties. The club, emmer, and common wheats varied in their degree of infection from heavy to moderate, and were more resistant than the durum and poulard wheats. The Polish and spelt wheats were only moderately infected, and these are apparently more resistant than the common type. Einkorn seems to be the most resistant, since it was only slightly infected.

In order to determine the relative susceptibility of a few winter wheats grown in New York, ten varieties were planted in triplicate plots in the fall of 1921. In the bottom of each drill row in one of the three plots, an equal amount of pure culture of the take-all organism growing on sterilized wheat kernels was placed at the time of planting. At the same time an equal amount of take-all-infested soil and infested parts of wheat plants, respectively, were added to each row in the other two plots.

In July, 1922, when the wheat had matured, the plants of each variety were dug and data were taken on the percentage of infected and of healthy plants, and the degree of stunting. Then, for the healthy plants and for each of the three degrees of stunting, data were taken on the average number of heads per plant and the average weight and number of seeds produced per head. The average yield per plant was obtained by multiplying the average number of culms by the average weight of seeds produced per head. By this method it was hoped that reduction in yield by take-all would be determined for each variety. However, in most varieties few disease-free plants were found. Therefore it was impossible to determine accurately the reduction in yield for individual varieties. But the data when averaged for the ten varieties gave fairly accurate figures, and showed that the yield of a take-all-infected plant which is not stunted is reduced 50.2 per cent as compared with that of a healthy plant; that the yield of a plant which is stunted less than half of its normal height is reduced 90.77 per cent; and that the yield of a plant dwarfed to more than half its normal height is reduced 99.39 per cent. The percentage of plants of each variety in each of the above classes, and the average yield in grams of one hundred plants of each variety, are given in table 2. The data in this table show that there is a marked difference in the degree of susceptibility of various wheat varieties. This difference seems to be constant, since practically every variety held the same relative position of susceptibility in each of the plots. The experiment was repeated in 1923, with duplicated plots. The results substantiated those of 1922 concerning the relative susceptibility of the ten varieties previously used. The testing of additional varieties showed that P 1066, Kanred Ks. 2401, and Turkey, were similar to P 1068 in that they all were very susceptible. Black Winter Emmer showed about the same degree of resistance as Dawson, and Red Winter Speltz and White Beardless Speltz showed greater resistance than any of the other wheats.

TABLE 2. RELATIVE INJURY AND YIELDS OF TEN WHEAT VARIETIES

	Per cent of plants infected with take-all			Per cent of plants not infected	Average yield of 100 plants (grams)
	Plants less than $\frac{1}{2}$ normal height	Plants $\frac{1}{2}$ to $\frac{9}{10}$ normal height	Plants of normal height		
Forward	11.0	21.1	66.7	1.2	135.4
Dawson	21.2	14.4	57.3	7.1	118.0
Red Wave	11.2	21.5	61.9	5.4	103.6
Golden Cross x New Columbia	23.7	22.8	49.7	3.8	112.9
Gold Coin (No. 6 Jr.)	22.3	28.1	45.4	4.2	92.3
Currells Prolific x Fultz	38.7	18.1	43.2	....	70.4
Fulcaster	23.3	22.0	53.2	1.5	74.78
Red Rock	39.1	18.9	40.8	1.2	68.50
Cosgrove	32.8	41.4	23.9	1.9	40.40
P 1068 Kanred	47.4	23.0	25.4	4.2	30.00
Average	27.07	23.13	46.75	3.05	84.63

Data based only on the percentage of plants escaping take-all infection are apparently worthless, since under favorable conditions nearly all plants become infected before reaching maturity. The varieties that yield the highest are invariably those in which the largest percentage of the infected plants are able to develop to normal size. In the varieties previously mentioned it will be noted that the harder wheats are the more susceptible and that there is seemingly no difference in susceptibility between red and white wheats.

THE DISEASE

NAMES

The name *take-all* has been generally applied in Australia, England, and other English-speaking countries to the disease under consideration. In the early stages it is called *yellow-leaf disease* and *take-all*. In later stages it is called *white heads*, *wit-roest*, *stand dead disease*, *white wilt*, *habit*, *wilt*, and *smoky*. In Europe somewhat similar diseases are frequently referred to as *foot rot*, *black foot*, *black stem*, *stalk disease*, *foot disease*, *root disease*, *black leg*, *straw blight*, *pietin*, and *pietin du ble*, but these are not to be confused with take-all.

HISTORY AND RANGE

Early references to root diseases of cereals are of little value, since it is impossible to determine whether take-all or a similar disease is meant. The take-all organism was first described by Berkeley and Broome (1861) from specimens found at Batheaston, England, on *Aira caespitosa*. Saccardo (1875) recorded the fungus from Italy on *Agropyron* sp. and *Cynodon* sp. In 1878 he reported it from France on wheat. It was again found in England in 1884 (Anonymous, 1913), and was next reported from France by Prillieux and Delacroix (1890). In 1901 it was recorded from Japan by Hori (1901), who stated that the disease had been present there since 1890 or 1891. McAlpine (1904) reported the disease as widespread in Australia at that time, and stated that it had been known there since 1852. Lindau (1908) records the presence of the disease in Germany and Holland. Dombrovski (1909) states that take-all occurs in Russia and Denmark, and Mangin (1914) records Belgium and Portugal as having take-all-infected cereals. The disease is reported by Waters (1920, a and b) as having been present in New Zealand for many years. It has been reported also from the Union of South Africa (Anonymous, 1922 a), where it was first found in 1904. In December, 1923, Dr. W. P. Fraser informed the writer that take-all had been found during the previous summer in a field of Marquis wheat in Saskatchewan, Canada.

Take-all was first reported from the United States in 1920 (Kirby and Thomas, 1920). In August of the next year, Humphrey, Johnson, and

McKinney (1921) called attention to reports of its appearance in Arkansas and Oregon. The writers state also that a "similar" trouble, of which investigations have indicated "a difference cause," has appeared in Virginia. Later, in a numeration of the various collections of take-all which have been made, McKinney (1923) alludes to the fact that the material collected in Virginia was in reality take-all, perithecia having apparently been discovered subsequently by members of the staff of the Office of Cereal Investigations, United States Department of Agriculture. In 1922 take-all was reported from Indiana and from additional localities in New York (Kirby, 1922), and it was shown that the disease was present in Kansas.[2] In 1923 specimens of the fungus which were collected in Tennessee in May were identified by the writer.[3] In addition the disease was reported from California (Mackie, 1923), and during July of 1923 Dr. J. A. Clark informed the writer that a considerable area of wheat infested with take-all had been found in North Carolina.

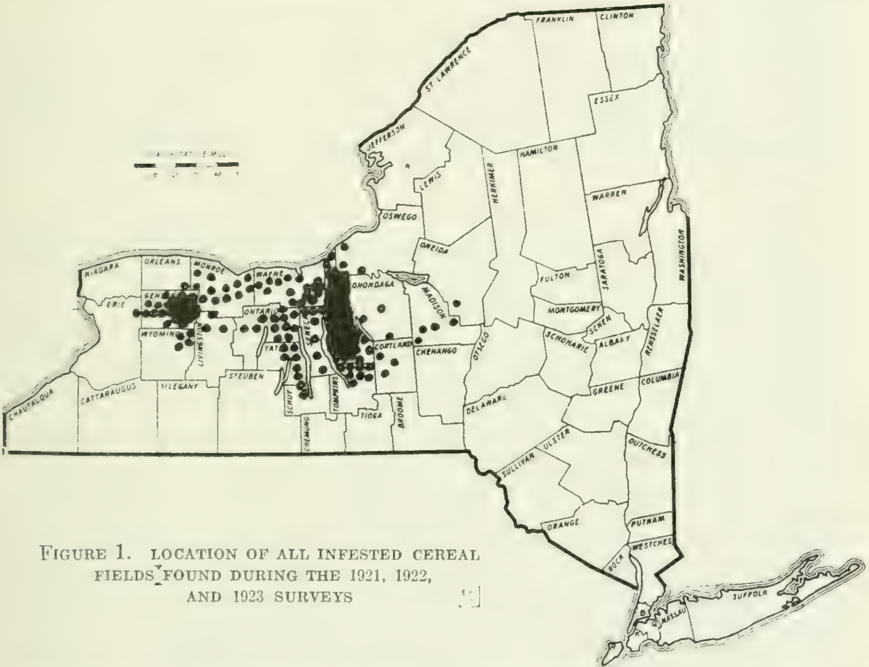

FIGURE 1. LOCATION OF ALL INFESTED CEREAL FIELDS FOUND DURING THE 1921, 1922, AND 1923 SURVEYS

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[2] Reported to the writer in a letter from R. P. White dated 1922, also by cultures which were sent to the writer in May, 1922.

[3] These specimens were sent to the writer by Dr. C. D. Sherbakoff on May 21, 1923.

Surveys were made in 1921, 1922, and 1923 in cooperation with the Plant Disease Survey of the United States Department of Agriculture, to determine the distribution of take-all in New York During those three years the surveys extended over fifty-seven of the sixty-two counties in New York, and included all sections where spring or winter wheat is commonly grown. The location of all take-all-infested winter-wheat fields found during these three surveys is shown in the accompanying map (figure 1).

Plants affected with take-all were found in 205 out of the 538 winter-wheat fields surveyed. The 205 infested fields were located in eighteen counties in the west central part of the State. This infested area seems to have definite boundaries, and coincides closely with the principal winter-wheat-producing area of New York. Within these eighteen counties, 48 per cent of the fields surveyed during the years 1921 to 1923 were found to be infected.

ECONOMIC IMPORTANCE

Take-all is one of the most serious diseases of winter wheat in the countries where it occurs. Its importance varies from year to year, depending at first on the climatic conditions; but later, when an area is once infected, the importance of the disease seems then to depend largely on whether or not rotation is practiced. Continuous cropping always results in increasingly greater losses; for example, in the Union of South Africa, where this is the prevailing practice (Anonymous, 1922 a), take-all results in an enormous amount of damage, and losses up to 100 per cent are reported. Hardly a field is free from the disease and it spreads rapidly. It is said that wheat-growing in the infested area there will soon be a thing of the past unless rotation is adopted. In Australia, where crop rotation is the exception and not the rule, many fields are almost entirely useless for wheat; and Mackinnon (1920) states that take-all reduces the average yield of Australian wheat fields from 12 to 15 per cent in certain years, although the average reduction for all years is probably not more than 7 per cent annually. In the United States, Stakman (1922) says that at Hillsboro, Oregon, the loss is estimated at one-third of the crop, and it is further reported (Anonymous, 1922 b) that in the same year, in that State, a considerable number of fields were so damaged as not to be worth threshing. In New York where rotation is usually practiced, the loss in the infested area varies from 0.3 to 2 per cent, although losses as high as 20 per cent have been observed in individual fields where rotation was neglected.

This disease causes a marked dwarfing of all parts of the wheat. The effect of the different degrees of dwarfing on the varieties of winter wheat grown in duplicate plots at Ithaca are shown in table 3. With increased severity of the disease as indicated by the degree of dwarfing, there is a proportionate decrease in the number of heads per plant, the number of

TABLE 3. EFFECT ON YIELD OF TEN VARIETIES OF WINTER WHEAT GROWN IN TRIPLICATE PLOTS RESULTING FROM THE DIFFERENT DEGREES OF DWARFING CAUSED BY TAKE-ALL

Class and description	Average number of heads per plant	Average number of seeds per head	Weight of 100 seeds (grams)	Average yield per plant (grams)	Average per cent of reduction in yield caused by take-all
1 Plants with no take-all	3.43	25	3.45	2.875	0
2 Plants affected with take-all. No reduction in height	2.65	23.6	2.295	1.431	50.2
3 Plants affected with take-all. Somewhat stunted, but never less than one-half the height of healthy plants	1.75	13.7	1.11	0.266	90.77
4 Plants affected with take-all. Stunted so that they are less than one-half the height of healthy plants	0.43	6.4	0.637	0.0176	99.39

seeds per head, the weight of the seeds, and the yield per plant. The reduction in weight of the seeds means that the seeds from wheat plants which were stunted by the disease will be so shriveled that they will be worthless as grain and most of them will be so light as to be blown out with the chaff in threshing. In addition to the reduction in the grain yield, there is a very marked degree of reduction in the amount of straw produced. This reduction in the yield of straw was apparently as high as 20 to 25 per cent, on an average, for all affected wheat plants.

The amount of reduction in the average number of heads, the number of seeds produced, the weight of the seeds, and the yield per plant, did not vary appreciably between the plants of different varieties which were in the same class as to dwarfing or having the same degree of infection, and the difference in the amount of loss between two fields or varieties was due to different percentages of plants occurring in the four classes. Class 2, in which the affected plants were not stunted, contained 46.75 per cent of all the plants. Since there was 50.2 per cent reduction in yield in this class, the total theoretical yield which would have been realized if all the plants had been free of take-all was reduced 23.4 per cent. In the same way, for the 23.13 per cent of all plants in class 3, where the affected plants were stunted not to exceed one-half the height of take-all-free plants, the reduction in yield was 20.9 per cent; and for the 27.06 per cent of all plants in class 4, where the affected plants were less than one-half as high as normal plants, the reduction was 26.90 per cent. Thus this disease caused an average reduction in yield in the ten varieties of winter wheat tested, of approximately 71.2 per cent. Since plants were not observed to die before the culms started to shoot up, the higher yield of plants recorded as disease-free seemingly was not due to the presence of fewer plants in a given area. In fact, there was evidence to show that the disease-free plants growing in the inoculated rows yielded less per plant than did the plants growing in check rows, where all the plants were free of disease. Thus it seems that the recorded loss due to this disease is probably too low rather than too high.

The 1922 survey in New York showed that 60 per cent of the infected plants in all fields examined were in class 2, 35 per cent in class 3, and 5 per cent in class 4. In applying the same rates of reduction in yield, the average loss in the infected plants would be 66.8 per cent. Since 1.1 per cent of the plants in infested fields were diseased, the loss in these fields was approximately 0.71 per cent.

SYMPTOMATOLOGY

The take-all disease affects the roots, the culms, the leaves, and the heads of its hosts, producing definite and characteristic symptoms. Signs[4] of the disease appear on the roots and on the lowest two or three internodes of the culms.

Symptoms

On roots.— Brown necrotic lesions appearing on the roots are the first symptoms of this disease. Such lesions were noticeable on the roots of winter wheat collected from a field on March 21, 1923, and the causal fungus was isolated from several of them. These lesions may completely or only partly surround the root. With the advent of warm spring weather the lesions increase in number, so that by the time the plant enters the jointing stage and sends up tillers (usually May 10 to 20 at Ithaca, New York) the greater number of the roots of badly infected plants are brown and dead and nearly all of the living roots have one or more lesions.

The killing of the roots progresses from the older to the newer ones, and to offset this loss the older roots continue to send out an abnormal number of branches which give them a woolly appearance (Plate II, A). New roots are often sent out from a point on the crown immediately above that of the emergence of the old ones. The killing of the roots weakens them to such an extent that a slight pull will break the plant off at or near the crown (Plate 1, B). Because of the fasciculation of the roots a considerable amount of soil is often pulled up with them, giving them a characteristic clubbed appearance.

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[4] Glossary. *In* Laboratory outlines in plant pathology, by H. H. Whetzel, L. R. Hesler, C. T. Gregory, and W. H. Rankin. 1916.

Under greenhouse conditions the take-all pathogene has been observed to cause lesions on the roots of wheat seedlings within fourteen days after planting, and to cause the death of the seedlings within another week.

On culms and heads.— The first symptoms to be observed in wheat plants under field conditions appear immediately after the plants have entered the jointing stage and when they are from six to ten inches high. These symptoms consist of a yellowing of the leaves and a dwarfing of the plants. (The earliest date of observing these symptoms at Ithaca is May 22.) The plants that are most severely affected soon die. By flowering time the diseased areas in the fields are strikingly characterized by the whitish color and the stunted stature of the plants contained in them. These areas are often 15 feet or more in diameter and are usually circular in outline. They contain from one to many plants. Their edges often overlap in badly infected fields, so that one may walk across such a field without leaving them. In some fields the disease occurs for several feet along the drill rows. The individual plants are usually dead and ashy white in color, and in the center of large areas are either killed out or dwarfed to a few inches in height. Most of the diseased plants are dead by flowering time, and on an average not more than one head per plant is developed.

The whitish color of the plant suggests the name *white heads* which is used by some writers to designate the later stage of this disease. A characteristic symptom is the marked reduction of the normal number of tillers, many of which die soon after their formation.

There is no striking change in the symptoms from the time of flowering to harvest. However, the diseased leaves and culms may become sooty due to the growth of saprophytic species of Cladosporium and Mucor. The " white head " stage only was observed in rye. Rye shows no chlorotic symptoms until after flowering, and therefore exhibits little or no stunting and only a moderate shriveling of the kernels.

The symptoms in barley as reported by Hori (1901) in Japan differ from those of wheat in that they often appear within thirty to forty days after the winter barley is sown. This stage is spoken of as the *yellow-leaf*. The other symptoms on barley are essentially like those on wheat.

On *Agropyron repens* the chlorosis and dwarfing were not conspicuous, but many lesions were found on the perennial rootstocks.

Signs

The first signs appear very soon after the chlorotic symptoms (May) and consist of a mat, or plate, of brown mycelium which extends from the root upward for several inches above the crown. The mycelium is in the leaf sheaths and between the culm and the inner leaf sheath, where it occurs as a plate-like mass. It causes a discoloration of the first and second internodes, forming a dark brown or black zone at the base of the diseased culms. Perithecia are formed during June and July in comparatively large numbers on the stems and in small numbers on the roots. After the death of diseased plants, or rarely before, the beaks of the perithecia may protrude through the outer leaf sheath.

In rye the plate mycelium is less pronounced and fewer perithecia are developed than in wheat, while on the susceptible wild grasses a definite plate-mycelium is practically always lacking.

Comparative symptomatology

True take-all differs in its signs and symptoms from the so-called "take-all" of Humphrey, Johnson, and McKinney (1921) reported from Madison County, Illinois, in that the latter disease exhibits hyperplastic instead of hypoplastic symptoms, there being an excessive production of culms in the Illinois disease; moreover, perithecia are wanting. Take-all differs also from the European foot-rots, the causes of which are usually not given by most investigators, though variously attributed to one or more of the following fungi: *Ophiobolus herpotrichus*, *Leptosphaeria herpotrichoides*, *L. culmifraga*, and *Fusarium* sp. Dr. D. Atanasoff states[5] that the European foot-rot is connected with a pathologic condition of the cereals which has nothing in common with the Australian or the American take-all. The European foot-rots are characterized by a wilting, breaking, and bending-over of fully grown plants, usually single plants or culms that have rotted bases. He says that affected plants are usually uniformly scattered throughout the field. Foex (1919 b) differentiates between the symptoms of take-all and the Leptosphaeria foot-rot, which is more characteristic of the European type of foot-rot, in that the take-all pathogene attacks the base of the stem and the underground parts, causing atrophy and premature dying, while *Leptosphaeria herpotrichoides* causes lodging and produces fruiting bodies higher up the stem.

ETIOLOGY

Nomenclature

The causal organism of the disease is *Ophiobolus cariceti* (B. & Br.) Sacc. In a previous paper (Fitzpatrick, Thomas, and Kirby, 1922) the evidence indicating that *Ophiobolus graminis* Sacc. and *Sphaeria cariceti* B. & Br. are the same organism has been presented.

Pathogenicity

Attempts to prove the pathogenicity of the fungus are reported by Delacroix (1901), Mangin (1902), and McAlpine (1904), each of whom inoculated growing wheat with diseased straw or spore suspensions, obtaining typical symptoms of the disease in every case. The first recorded inoculations with the fungus from pure culture were made in New Zealand by Waters (1920, a and b), on wheat seedlings grown from sterilized seed on sterilized soil in test tubes. Inoculated plants died in from twenty-eight to thirty-six days from the date of inoculation, while control plants were in good condition after fifty-eight days. Inoculated culms of wheat plants growing in pots did not become infected.

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[5] In a letter to the writer dated March 9, 1923.

In the present investigation the relation of *Ophiobolus cariceti* to take-all has been clearly established. The fungus was found regularly associated with the disease in each of the 205 infested fields of winter wheat observed during the four years while this investigation was in progress.

The fungus was isolated by the following methods: Bits of the host tissue bearing one or more perithecia were immersed in a 1-2000 mercuric chloride solution for from one to two minutes and then placed under a binocular, where by means of needles individual perithecia were freed from foreign matter. The perithecia were then crushed in a large drop of sterile water, and this drop was diluted by the addition of sufficient sterile water so that if individual small drops were taken each would contain approximately one ascospore. Drops of this suspension were then atomized on, or several drops were spread over, the surface of dextrose agar[6] in petri dishes. After twenty-four to forty-eight hours, when the petri dishes were examined under the low power of the microscope, the ascospores had started to germinate and single ascospores were transferred with a needle to the surface of agar in petri dishes. During the latter part of the investigation another method[7] was used to obtain cultures from single spores. This consisted in placing individual drops of the ascospore suspension on small pieces of sterile cover slips. The drops on the slips were examined under the microscope, and only those in which the drop contained one ascospore were transferred to the surface of agar in petri dishes. Subsequent examinations were made daily, and where no contamination developed, and the ascospore germinated and produced a colony, a mycelial tip was taken. A third method of isolation consisted in the planting of small bits of diseased host tissue in agar in petri dishes, and the subsequent transfer of single mycelial tips to agar in test tubes.

The first and second methods were used when viable ascospores were obtainable.

Isolation of the fungus from tissue plantings is difficult, because of its slow growth and the occurrence of other more rapidly growing organisms in the tissue. Nevertheless the fungus was isolated from fifty tissue plantings, and more than one hundred and ninety ascospore dilution cultures were made, in the comparative study of the fungus in pure culture from many sources. The growth of this fungus on various media is in general of two types, examples of which are as follows:

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[6] Formula; 100 cubic centimeters water, 20 grams agar, 2 grams dextrose.

[7] This method, as well as the formula for the agar, was suggested to the writer by Dr. H. F. Thomas.

Type I: On 0.2-per-cent dextrose agar the growth (Plate III, A and B) reaches 2.5 centimeters in diameter after seven days, and seldom exceeds from 5 to 7 centimeters in thirty days or longer. The mycelium after seven days of growth is submerged or wholly appressed. The colony is translucent and is made up of feathery, rhizomorphic strands resembling the feathery branchings of some mosses. After thirty days the appearance of the colony is nearly the same as at the end of seven days, except that it has become in color a deep neutral gray[8] and hyphal strands have appeared near the center. The rhizomorphic strands are appressed to the surface or submerged. Single hyphae radiate toward the edge of the culture. The cells of the hyphae are from 16 to 45 μ long and from 4 to 5.5 μ in diameter, and contain from 10 to 25 guttulae. The cell walls are hyaline. Branches arise near the tip of about every eighth to tenth cell, or at a distance apart of about every 150 to 250 μ. The cells of the branches are much more variable in size and shape than those of the main hypha, ranging from knob-like cells with a diameter of 5μ, such as occur at the end of a few hyphae, to cylindrical cells resembling those of the main hypha.

Type II: On potato agar (5.4 pH) the growth (Plate III, E and C) reaches 7 centimeters in diameter at the end of seven days, and covers the plate (9 centimeters) in from eight to nine days. After seven days of growth the mycelium is hyaline, and is submerged or wholly appressed. The individual colony is abundantly and distinctly flexuous; its margin is even but is not marked by a definite line, appearing finely frayed. At thirty days the mycelium of the colony has assumed a more pronounced aerial habit, rising to a height of 2 millimeters and growing on the sides of the petri dish. The aerial growth causes the surface to appear as if covered with a silvery bloom. The colony ranges in color from a neutral gray to a deep neutral gray. The surface of the medium is thickly covered with tortuous, mixed, ribbon-like, rhizomorphic bands of hyphae. These bands are made up of from two to eight strands of parallel hyphae. The cells of the individual strands are cylindric, and have thick, dark neutral gray walls; they are 75 to 260 μ long and from 4 to 6.5 μ in diameter (averaging 184 by 5 μ). The contents are hyaline and sparingly guttulate. Nearly every cell in the strands sends forth a branch at its tip. The cells of the hyphal strands closely resemble those of the plate mycelium on the host (Plate III, C). The branches terminate in thin-walled hyaline hyphae, the individual cells of which contain from 10 to 30 guttulae. The cells are very variable in shape and size, ranging from 15 to 50 μ long and from 2 to 6 μ in diameter. Anastomosing is observed frequently.

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[8] Ridgway, Color standards and nomenclature.

Type I, which is characterized by the radiate habit of the strands, occurs only on the 0.2-per-cent dextrose agar. Type II, which is characterized by the strands intermixing on the plate and forming ribbon-like strands of parallel hyphae, occurs when the fungus grows on the following agar media: potato, cornmeal, bean, oatmeal, prune, nutrient, Czapek, crushed wheat (Kirby, 1922), and 2- and 5-per-cent dextrose. On crushed wheat agar aerial growth is much more abundant than on any of the other media. Zonation does not occur on potato agar with a pH of 5 to 6, but it does occur and becomes increasingly pronounced as the pH value decreases or increases.

The writer has been able to develop perithecia in culture, both in petri dishes containing crushed-wheat agar and on sterilized sweet clover and wheat stems. Different monosporous isolations of the organism showed a marked difference in their capacity to develop perithecia. Some of these were greatly stimulated in their development of perithecia by being grown in apposition with other monosporous cultures. From a considerable number of these isolations perithecia were not obtained except when they were grown in this manner. However, this was not true of all the cultures studied, since some of them were found to produce perithecia without stimulus of a second strain. It was believed in the earlier part of the work that the majority of the strains of the fungus exhibited a true heterothallism (Kirby, 1923). Subsequently strains have been found which are not identical in their cultural reactions with either of the original plus or minus types. Further investigation will be necessary to determine whether or not the strains of this fungus can be segregated into definite sex types.

The characteristic signs and symptoms of the disease have been produced by inoculations of pure cultures of the fungus into healthy plants. In several series of inoculation experiments, the soil of 457 pots contaminated with pure cultures of the fungus at the time of planting were variously sown with wheat, rye, barley, or other species of grasses. Within four months after planting, characteristic symptoms and signs appeared on plants in all the pots, while the plants in 212 pots planted under the same conditions excepting that no inoculum was placed in them never showed signs or symptoms of take-all.

In a field which was known to be free of take-all, 64 rod-rows of winter wheat of different varieties were inoculated in the fall of 1921 with pure cultures of the fungus, and in every row the same characteristic signs and symptoms of the disease were produced as have been found in all infected fields observed in New York. In this test field, the same symptoms and signs appeared when 47 rod-rows of winter wheat were inoculated in the fall of 1921 by placing diseased straw brought from other fields with the seed at planting time. Further take-all symptoms and signs appeared when 45 rod-rows of winter wheat were inoculated with pure culture and diseased straw in the fall of 1922. In the same tests, 209 rod-rows of winter wheat growing more than one foot distant from inoculated rows, which received no inoculum, produced no signs or symptoms of take-all. Typical symptoms of the disease were produced when the roots of fifty sterile wheat seedlings were inoculated with a pure culture of the organism growing in large individual test tubes. At the same time, twenty uninoculated seedlings growing under the same conditions showed no symptoms of the disease.

The fungus was reisolated at least twelve times from the lesion areas on plants that had been inoculated with pure cultures, but it was never isolated from bits of the host tissue taken adjacent to the discolored area. The reisolated fungus agreed in every essential with that in the original cultures. Furthermore, when used as inoculum at planting time, these cultures gave typical symptoms of take-all on the growing wheat plants in the field. There can be, therefore, no doubt that the fungus *Ophiobolus cariceti* is the cause of the take-all disease.

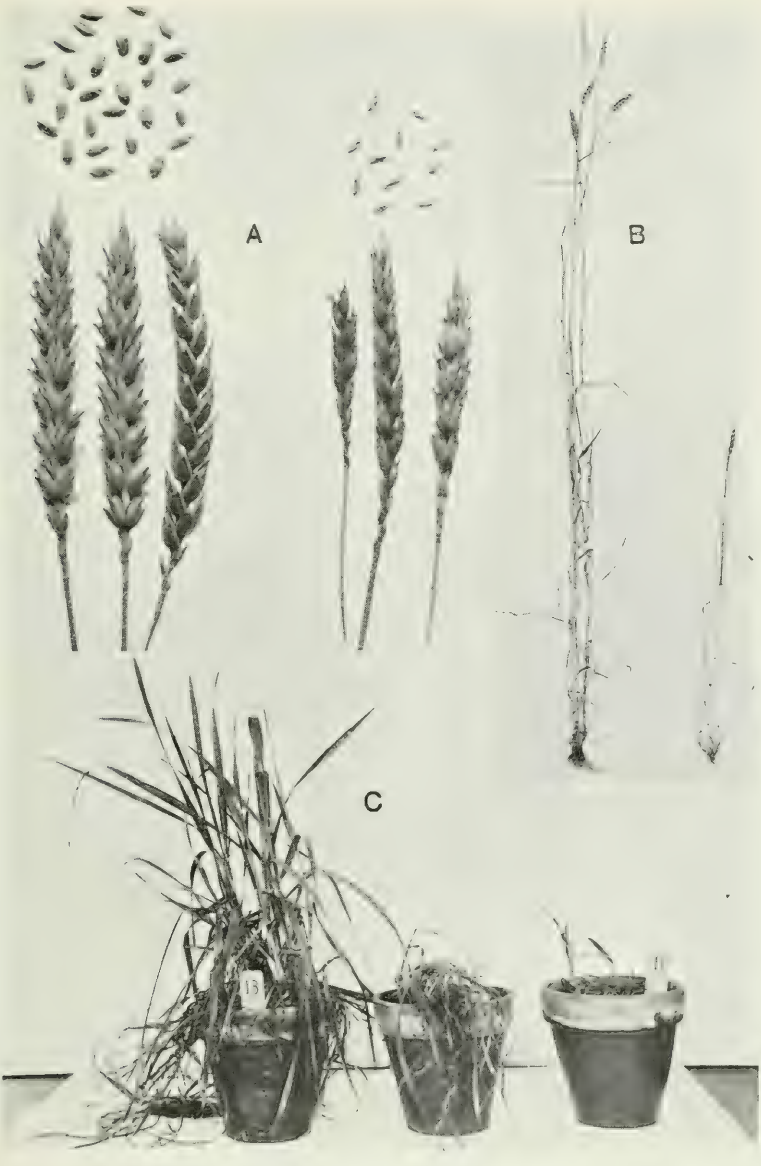
Life history

The life history of this fungus exhibits no definite separation into primary and secondary cycles. Infection appears to take place at any time during the year when moisture and temperature conditions are favorable. It appears to be initiated usually by ascospores, but there is evidence that the fungus may spread from plant to plant by mycelium growing through the soil.

Pathogenesis

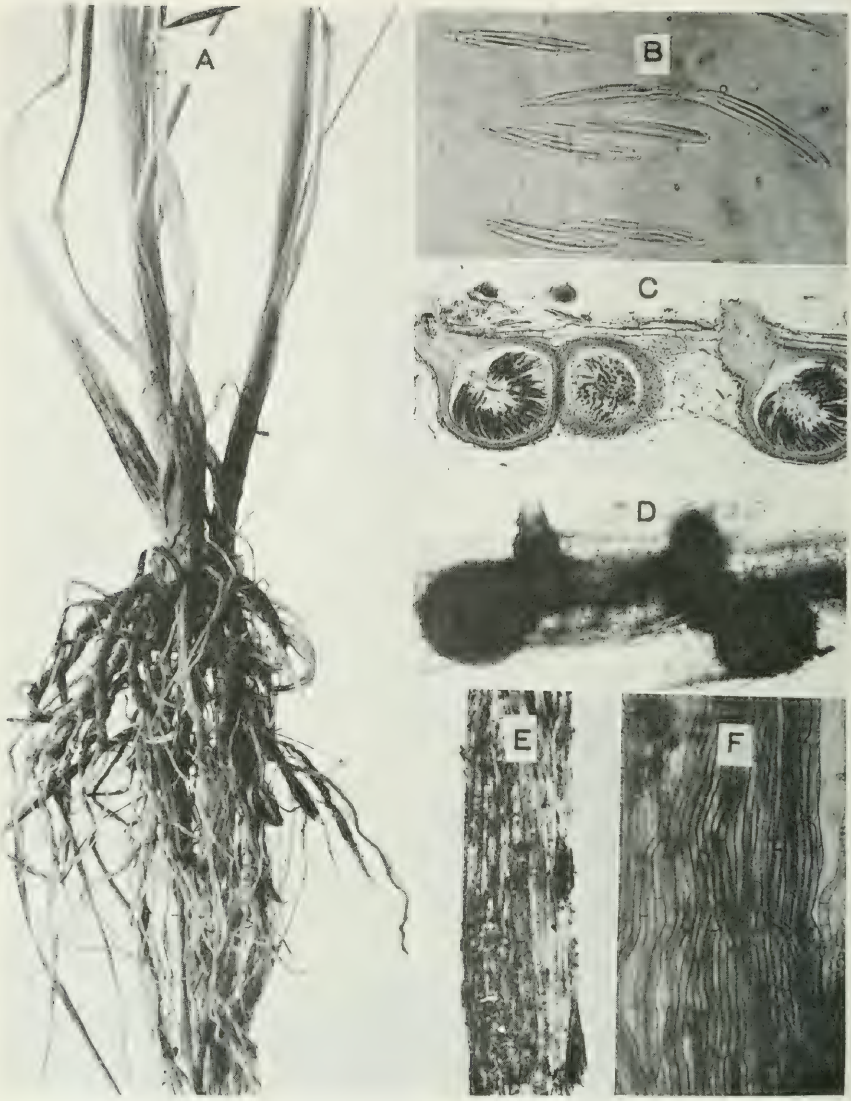
Inoculation.— The chief sources of inoculum are the lower internodes and the roots of diseased plants. Upon these perithecia are formed during a period of four weeks preceding harvest. The rootstocks of *Agropyron repens* have been observed to harbor mycelium of the take-all pathogene for twelve months. Wheat planted among these rootstocks soon showed signs and symptoms of the disease. Thus mycelium-harboring grasses are another important source of inoculum. Winter wheat planted in September in soil removed from infested wheat fields the previous spring has, in both 1920 and 1921, become infected. In this case mycelium harbored in the soil during the summer probably acted as the inoculum in the fall. Concerning the source of this inoculum, Spafford (1917) states that it appears to come wholly from the soil, particularly from straw of affected plants left in the field.

Field observations have indicated that the take-all fungus may be harbored with the seed to a very small extent. However, repeated plantings of seed from diseased plants in the greenhouse have given negative results. In an effort to ascertain whether the pathogene is transmitted with the seed, the following test was made in the fall of 1921 on land



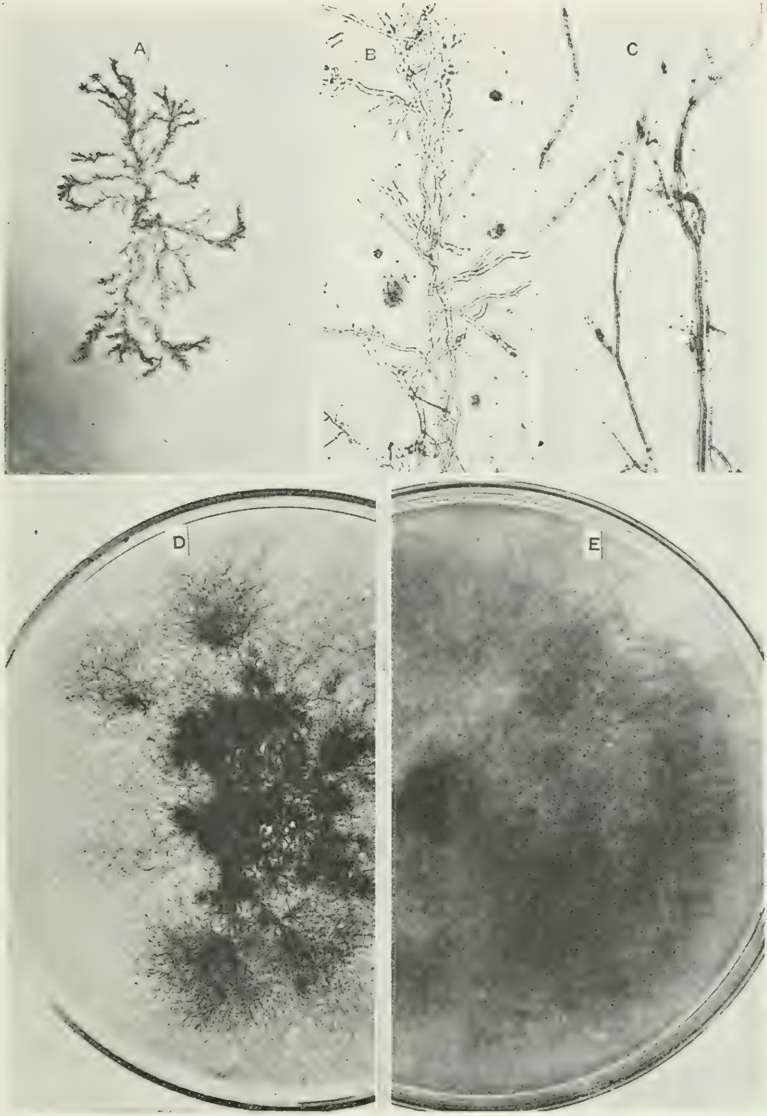
HEALTHY AND TAKE-ALL-AFFECTED WHEAT PLANTS

A, (left) Healthy wheat heads, (right) take-all-affected wheat heads. B, (left) Healthy plant with five heads, height 40 inches; (right) diseased plant with one shriveled head, height 18 inches. C. Plants after four months growth: pot 13, check; pots 11 and 12, diseased straw mixed with soil at planting time



SYMPTOMATOLOGY OF THE DISEASE, AND MORPHOLOGY OF THE PATHOGENE

A, Base of wheat plant, showing lesions on culms and on roots. B, Asci and ascospores (× 130.3). C, Longitudinal section of a wheat plant showing structure and contents of the perithecia (× 39.4). D, Two perithecia developed below the outer leaf sheath; the oblique beaks illustrate one of the most characteristic features of the species (× 24). E, The lower part of a culm of a wheat plant infected with take-all, showing protruding beaks of the perithecia (× 3.3). F, A part of the mycelial plate found about the base of the culm (× 130.3)



THIRTY-DAYS-OLD CULTURE OF OPHIOBOLUS CARICETI ON 0.2-PER-CENT DEXTROSE AGAR AND ON POTATO AGAR

A, Culture on 0.2-per-cent dextrose agar (× 8.9). B, Characteristic rhizomorphic strands of mycelium as found on 0.2-per-cent dextrose agar (× 450.7). C, Characteristic ribbon-like rhizomorphic bands of mycelium as found on potato agar (× 450.7). D, Culture on 0.2-per-cent dextrose agar. E, Culture on potato agar

which was known to be free from the organism. Twenty-one rod-rows of winter wheat were planted with seed obtained by hand-threshing diseased plants collected from all the infested fields surveyed in 1921. Special care was taken in the threshing to see that no part of the culms became mixed with the seed. No symptoms nor signs of take-all were observed in any of these rows. In addition to this test, 77 rod-rows of winter wheat were planted with seed that came from a badly diseased field, the grain of which was threshed by the usual methods. One or two diseased plants appeared at one place in these rows. This seemed to indicate that the pathogene may be carried with seed in which bits of diseased culms have become mixed.

It appears, however, that the ascospores constitute the inoculum for the vast majority of infections. The perithecia in which they are developed are usually produced in great numbers on wheat, on barley, and on many grasses. These minute dark brown to black, rostrate fruit bodies (Plate II, C and D) are most commonly and abundantly developed beneath the leaf sheaths of the lower internodes. They occur also on the roots in the thick wefts of fine rootlets developed above the diseased crown. They are gregarious or scattered. When young the perithecia are hidden, but at maturity their prominent beaks protrude through the leaf sheath.

These perithecia contain numerous elongate clavate asci, each containing eight spores (Plate 11, B). These ascospores are long, slender, and hyaline, and are continuous when young but at maturity are 5–7-septate. They measure 60 to 107 by 3 to 3.75 μ. They do not usually become fully mature until early autumn.[9]

In the examination of several thousand individuals it has been noted that soon after formation the perithecium contains a few apparently mature and many immature ascospores, and that during the following nine months it always contains a proportion of immature ascospores. Hori (1901) states that in Japan the ascospores germinate in June and July. McAlpine (1904) reports that in Australia they germinate during November, and Robinson (1907) finds them capable of germinating immediately after formation if sufficient moisture and air are present. However, no investigator has reported on a careful study of their longevity.

With a view of ascertaining the length of the period during which ascospores will germinate, field material was collected and stored under field conditions in 1920, 1921, and 1922. During those three years attempts were made to germinate the ascospores nearly every month. The results of these tests show that ascospores formed in June and July have in some years failed to germinate and have in other years germinated to a small extent (less than 5 per cent) in July and August of the same year. In September the germination increased to about 25 per cent, and the maximum (50 per cent) was reached during October, November, and December. The percentage of germination fell off gradually during January, February, and March. The latest germination to be observed was in March and April, after which all the ascospores failed to germinate. Thus it appears that most of the ascospores come to maturity and are discharged soon after the winter wheat is planted in the autumn, at which time inoculation and infection usually occur.

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[9] A detailed description of the morphology of the perithecium has been given by Fitzpatrick, Thomas, and Kirby (1922:36–37).

The manner of ascospore discharge from the perithecium has been studied by Hori (1901), McAlpine (1904), and Waters (1920 a). These investigators all agree that the ascospores escape through the ostiole but that they are not shot forth into the air. McAlpine stated further that the asci are expelled one after another, and the ascospores are then set free in the presence of moisture. Waters (1920 a) pictures perithecia which have pushed out coils of mucilaginous matter carrying the asci through the neck with it. He states that this takes place in slightly moist air, that in very moist air a globose mass is formed at the ostiole, and that in water the moisture dissolves the mucilage and liberates the spores as the asci emerge from the ostiole. A dry mass of asci and ascospores has several times been observed by the writer to have formed at the ostiole, where it remained for several months if there was no rain to dissolve it. Apparently asci and ascospores are discharged only when there is sufficient moisture present to soak up the contents of the perithecium. Perithecia formed in pure culture have not been observed to liberate their spores until placed in water. Field observations seem to indicate that asci and ascospores are discharged during rainy periods, and that usually the splashing rain removes them before they can accumulate at the ostiole.

The date at which ascospores are mature and ready to be discharged may be determined by immersing perithecia from time to time in water, when, if mature, the ascospores will be exuded. Hori (1901) states that in Japan at least some spores are discharged within ten days after the crop is cut, or about June 23, and Waters (1920 a) states that in New Zealand perithecia put forth tendrils of spores in January (corresponding to July in New York). From observations made by the writer, it seems that the date of discharge is greatly influenced by the time of formation of the perithecia and the subsequent moisture conditions. Perithecia have developed as early as June 4 and have been observed discharging their ascospores within two weeks after their formation. It has been noted that perithecia seldom discharge all their ascospores, but retain a certain number which finally disintegrate during April and May following their formation. Ascospore discharge may begin as early as the latter part of June, but it probably does not reach its maximum until September and November, ceasing some time in March or April of the following spring.

It is clear that the behavior of the ascospores at the time of their discharge restricts the agents of dissemination almost completely to rain. To determine whether they could live long enough to be wind-borne, the following test was made. A suspension of ascospores and asci was prepared, small drops of which were placed on cover slips and allowed to dry. At definite time intervals after the drops had dried, water was again added and the cover slide was put over a Van Tiegem cell in an attempt to germinate the spores. The results were as follows: ascospores in unexposed controls germinated 49 per cent; free ascospores exposed for two minutes germinated 2 per cent; free ascospores exposed for three, five, ten, and thirty minutes, and one, two, and twelve hours, failed to germinate. Asci exposed for three minutes showed ascospores germinating in 40 per cent; those exposed for five minutes showed 35 per cent germination; those exposed for ten minutes showed 16.6 per cent germination; and those exposed for thirty minutes and for one, two, and twelve hours failed to show any germinating ascospores.

Hori (1901) states that spores are disseminated in the soil near the host and that wind may carry them for a short distance. Results of the present investigation indicate that ascospores, and to a certain extent asci, are so susceptible to drying that they cannot be transported in this manner. Besides the dissemination of single ascospores, the fungus may be carried as perithecia on host tissue. Waters (1920 a) states that infected fragments may be carried by animals, wind, and cultivating tools, and McAlpine (1904) believes that much of the dissemination of the fungus in parts of Australia is due to dust or wind-blown soil. Mycelium harbored on the rootstocks of perennial grasses may be scattered by cultivation.

Mangin (1899), Hori (1901), McAlpine (1904), and Waters (1920 a) have all stated that the roots serve as the infection court and that they were unable to get infection on the upper part of the wheat plants. The results obtained during the present investigation corroborate their findings.

Incubation.— In water cultures, ascospores germinate either when free or while still in the ascus. Germination begins in about ten hours, by the pushing-out of a germ tube from one or both ends and in some instances at points near the middle of the ascospore. A knob-like swelling, of a diameter of 5 to 8 μ, has been observed to develop at the end or along the middle of the ascospore. One or more germ tubes emerge from the distal end and the sides of this swelling.

Sickle-shaped microconidia, which seem to be functionless, may bud out from the ends and the sides of the ascospores. These microconidia are hyaline, measuring 5.5 to 8 by 1.5 to 2 μ. They are borne also on the ends of certain hyphae which function as conidiophores. Foex (1919 c) considered them sporidia and called them falciformers. McAlpine (1904) recorded their presence without giving any hint as to their function.

The germ tube, after its first appearance, grows rapidly and may reach a length of 55 μ and 300 μ in 24 and 48 hours, respectively. The germ tube is hyaline. Septa are not developed until during the second day's growth. Guttulae or fat globules are found in the germ tubes after the septa are laid down. These observations agree with those of Delacroix (1901), McAlpine (1904), and Hori (1901), except that Hori appears never to have observed germ tubes arising from the middle of the ascospore.

The effect of varying degrees of acidity on ascospore germination was determined by germinating ascospores in thirteen different solutions prepared with hydrochloric acid and sodium hydroxide and ranging in acidity from pH 3 to 10. The results of this test are shown in figure 2. The bimodal germination curve shows that ascospores of this fungus germinate through a smaller range of acidity than that in which growth takes place. The rate of growth of the germ tubes in the solutions of different pH values was greatest at pH 7 and was only slightly less at pH 8 (hyphae 180 μ long in 48 hours .

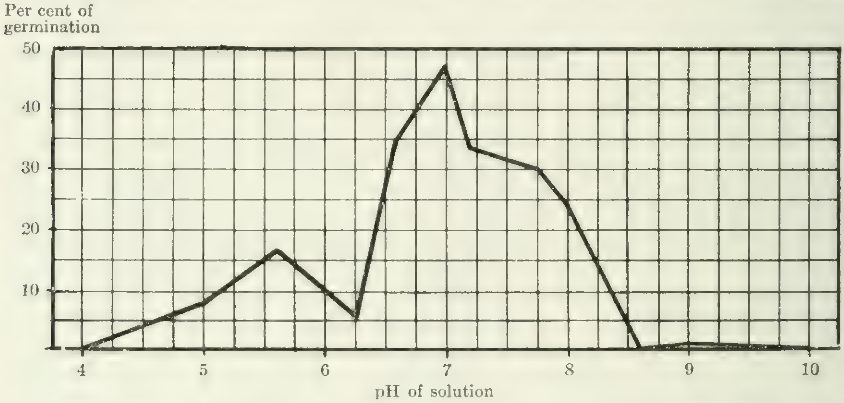

FIGURE 2. PERCENTAGE OF ASCOSPORES OF OPHIOBOLUS CARICETI GERMINATING AT VARYING DEGREES OF ACIDITY

Concerning penetration, Mangin (1899) reports that the germ tube creeps along the surface of the root hairs and soon pierces the membrane and enters the interior of the plant. In the present investigations, wheat plants growing in pure culture in test tubes on 0.2-per-cent dextrose and potato agars in petri dishes were inoculated with ascospores, and the growth and penetration of the germ tube were observed under the microscope. Approximately fifty inoculations were made in this manner, and it was noted that the germ tube of the pathogene grew along the surface of the roots and nearly always entered the epidermis directly. In one case a hypha formed a small appressorium on a root hair, and from this a penetration tube entered and passed down the root hair into the root; but in the other four cases of root-hair penetration observed, appressoria were not formed.

Infection.— In wheat plants growing on agar plates the first evidence of infection appeared seven days after the fungus had come in contact with the roots, and consisted of small, brown, necrotic spots. These spots enlarged so rapidly that within a period of from three to five days the entire root for a distance of one-quarter to one-half inch was dead and brown, all of the tissues being permeated with mycelium. Robinson (1907) states that in Australia young seedlings planted in diseased soil may be affected almost as soon as they appear above ground. McKinney (1922) reports that plants in the greenhouse show severe seedling blight and often die within fourteen days after sowing.

Under New York field conditions, symptoms of the disease have not been observed on the culms before May 20, but lesions have been found on the roots as early as March 19. The fungus has been isolated from such lesions.

The death of the roots reduces the supply of moisture and mineral nutrients to the plants. To overcome this, many fine rootlets are developed above the lesion. The plant may also send out new roots at its crown. These efforts of the plant to recover seem to be of little value when it is attacked while young, since the mycelium grows upward inside and outside of the roots to the crown, attacking and killing all roots with which it comes in contact. The mycelium enters the leaf sheaths at the crown, permeating the tissues.

The septate mycelium is of two general types, colorless and brown. The very young mycelium, and nearly all the mycelium within the culm and the roots, is colorless. This consists of rhizomorphic strands, the individual cells of which are cylindric, thin-walled, hyaline, and 10–30-guttulate. These cells are from 15 to 50 μ long and from 2 to 6 μ wide. The cells of the branches are very variable, ranging from knob-like cells with a diameter of 5 μ, to cells resembling the main rhizomorphs. The mycelium is abundant in the xylem tubes and grows intracellularly in all the other tissues.

By the time the mycelium has entered the leaf sheaths, the roots are usually so severely affected that the plant becomes yellow. Soon after this symptom appears, a mass of mycelium is formed between the inner leaf sheath and the culm. This mass is composed of hyphae which intermingle to form a plate, which usually adheres to the culm when the leaf sheaths are stripped away. The plate mycelium thus formed consists of flat, ribbon-like strands resembling somewhat compressed rhizomorphs, which run definitely parallel to one another. The individual cells of these strands are cylindric, and are from 20 to 260 μ long and from 3 to 6.5 μ wide. Their contents are sparingly guttulate and hyaline.

Saprogenesis

With the formation of the plate mycelium the plant usually succumbs, and thereafter the fungus leads a saprophytic existence. Perithecia are now formed on the inner side of the outer leaf sheath and in mycelial mats covering the roots. These leaf sheaths and roots are nearly always dead before perithecia are developed, and often the entire plant has died before the perithecia reach their full size. Fully developed perithecia have been formed on wheat in the greenhouse within eight weeks after planting of the seed, but in the field they have not been found before June 4. The ascopores which are discharged early in the summer are probably washed to the ground, where they germinate and produce mycelium which may live saprophytically in the soil on organic matter until the fall, when wheat is planted. The mycelium in the soil about the cereal or grass plants may also live saprophytically until fall.

The ascospores in the perithecia are only partly discharged at any one time, as already pointed out. Hori (1901) states that the ascospores may germinate and live saprophytically on the decayed culm until autumn, when the mycelium attacks the young cereal plants.

While the life cycle of this pathogene is usually initiated by ascospores, mycelium spreading through the soil also may infect healthy plants. Hori (1901), McAlpine (1904), Waters (1920 a), and others have noted that the infection occurs and seems to spread in circular areas. Spafford (1917) states that mycelium spreads from root to root by dark brown mycelial strands. During the present investigations it was noted that in infested wheat fields the margin of the spots killed by take-all was composed of a border, ranging in width from 6 inches to several feet, wherein the infected plants were not stunted and seemingly had been attacked late in their development. The 1921–22 plot gave ample opportunity for observing the spread of the pathogene through the soil, since inoculum was placed only at known points in the field. In these plots it was clearly shown that during the period of growth of winter wheat the mycelium could not have spread through the soil for a distance greater than one foot. Only a fraction of one per cent of the plants were killed in their later stages of growth at this distance from inoculum, consisting of pure cultures or diseased wheat straw, placed in the ground at planting time. Thus it seems that the fungus is able to spread through the soil for very short distances only and initiate new life cycles.

ECOLOGY

Moisture and temperature

There seems to be a direct correlation between such climatic factors as moisture and temperature, and the amount and severity of take-all. It is said (Anonymous, 1922 b) that climatic factors have a tremendous influence on the severity of the disease in Oregon, and that it appears probable that only in certain especially favorable seasons will the disease result in serious crop damage. Guerrapain and Demolon (1913) conclude that high autumn temperatures promote the infection of seedlings in France. Dombrovski (1909) and Mangin (1914) state that high humidity of the soil favors the disease. Robinson (1907) believes that a dry summer followed by a wet winter affords the most favorable conditions for fall infection. Brittlebank (1920) reports that water-logging of the soil before harvest, with a subsequent drying, favors the development of the disease.

Observations made during the present investigations seem to corroborate in general the foregoing statements regarding the effect of climatic factors. In most of the infested wheat fields examined there was little apparent difference in the amount of infection on high and low ground, although in a few fields the infection appeared to be much heavier on the lower and wetter soils. In the 1922 test plot an inoculated check row in water-logged soil had a higher percentage of badly diseased plants than did the rows on drier soil.

Varying climatic conditions which occurred during the springs of 1921, 1922, and 1923 brought about marked differences in the amount and severity of take-all. As shown in figure 3, the spring of 1921 was warm and wet during March and April, while May and June were exceptionally dry. Under these conditions there occurred a higher percentage of extreme stunting, combined with a premature dying of the infected winter-wheat plants.

The spring of 1922 differed from that of 1921 in that it was cooler or rather later, and in May, and especially in June, there was greater rainfall. Under these conditions about 40 per cent less plants were found to be diseased in the infested fields than in 1921. The plants that were affected died later and the amount of stunting was much less.

The spring of 1923 was abnormally late, cool, and dry, with the low rainfall continuing until harvest time. Under these conditions the disease was far less severe than in either of the two preceding years. This decrease in the severity of the disease is shown by the fact that in 1923 the disease appeared two weeks later than ever before, and 70 per cent less plants were found diseased in 1923 than in 1922; also, in 1923 there was less dwarfing of the affected plants than in any previous year.

Thus the weather conditions which seem to favor the disease are abnormally high temperature and rainfall in the early spring (March and April), followed by dry, hot weather from the period of jointing until harvest. Springs in which abnormally high temperature and rainfall occur seem to be less conducive to the development of the disease if during May and June the rainfall is abnormally high and the temperature is normal or below.

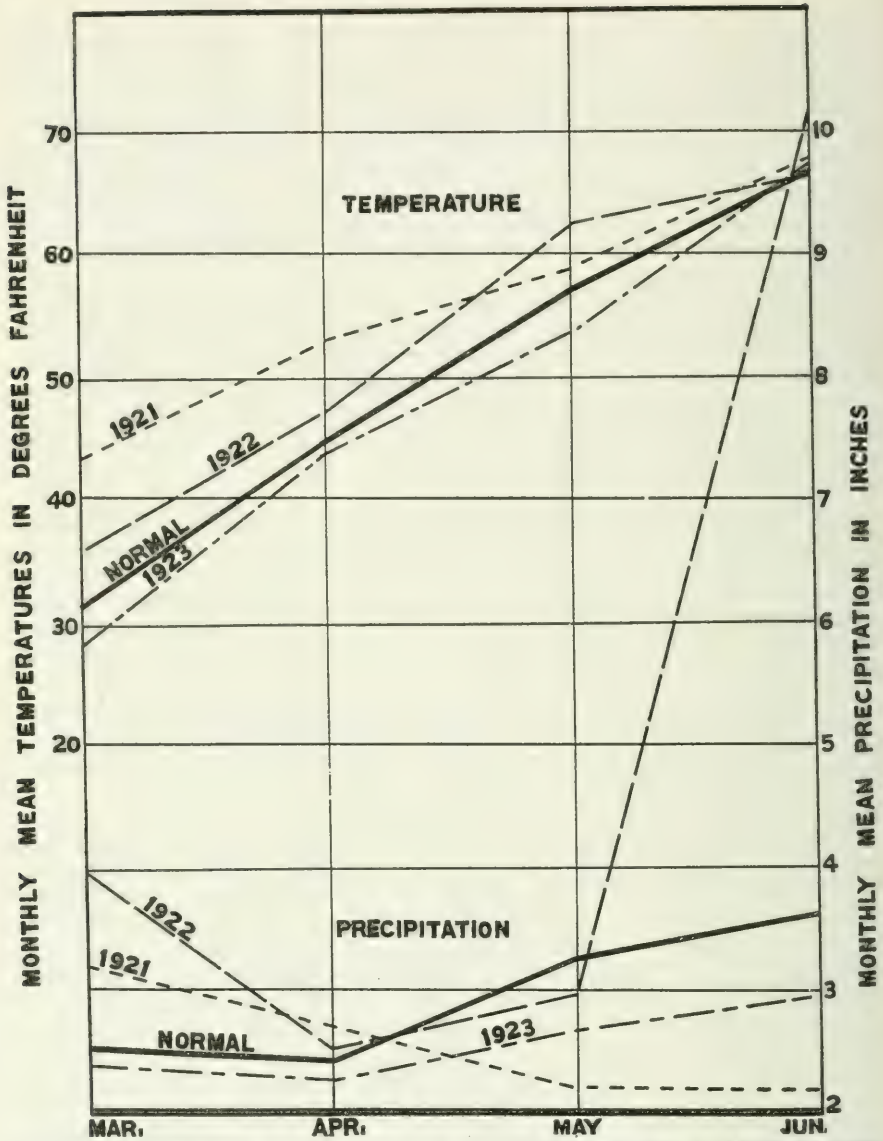

FIGURE 3. THE MONTHLY MEAN TEMPERATURES AND PRECIPITATION FOR FOUR SPRING MONTHS IN 1921, 1922, AND 1923, COMPARED WITH NORMAL TEMPERATURE AND PRECIPITATION

The mean in this data is the average of conditions at Ithaca, Syracuse, and Rochester

Abnormally low temperatures and rainfall in the early spring, such as occurred in 1923, seem to be very unfavorable to the development of the disease.

McKinney (1922) reports that the most favorable temperature for the fungus to attack wheat seedlings is near 22° to 24° C. This explains why take-all may be more destructive in the earlier-planted winter wheat. After the time of early wheat planting the temperature is often above 22° C., but after the time of the late planting the temperature seldom reaches this point.

Soil fertility and acidity

Several investigators have studied the relationship between the type of soil and the severity of take-all. Hartmann (1914) and Gray (1914) state that the disease is worse on sandy, light soils than on heavy, stiff, clay soils; while Hori (1901) believes that in Japan the disease is worse on clay or loamy clay than on sandy soils. Richardson (1911) states that take-all occurs on all types of soil.

Many workers have investigated the influence of various fertilizers and other substances on the development of the disease. Rosen and Elliott (1923) found that under Arkansas conditions commercial fertilizer almost completely prevented the disease while manure decreased the percentage of diseased plants to a considerable degree. Hori (1901), working in Japan, found that the intensity of the damage apparently bears a close relationship to the nature and amount of the fertilizer applied to the soil. The greatest damage occurred when an excessive amount of nitrogenous fertilizer was applied or when the three essential elements were unbalanced. Lime has generally been reported as greatly increasing the amount of disease (Hartmann, 1914, Brittlebank, 1920, and Rosen and Elliott, 1923). Superphosphate of lime has been reported as increasing (Brittlebank, 1920), as not checking (Mangin, 1902), and as decreasing (Anonymous, 1913) the growth of the fungus. Copper sulfate in some cases had no particular effect in the control of the pathogene (Foex, 1919 b), while in other cases it caused a reduction in the amount of the disease (Darnell-Smith, 1916, Foex 1919 b and Benoist and Bailly, 1922). Sulfuric acid and chloride of potassium resulted in a marked reduction in foot rot caused by *Ophiobolus cariceti* and *Leptosphaeria herpotrichoides* (Benoist and Bailly, 1922).

From these more or less conflicting reports there appear to be two factors which decrease the amount of take-all: first, the type of soil fertility which enables the plants to grow at a moderate rate, but not with a profuse vegetative growth such as occurs when an excessive amount of nitrogen is present; and secondly, an acid condition of the soil. To test the fertility factor in a preliminary way, the following experiment was run in the greenhouse. Twenty-five 5-inch pots were filled with clean soil and were inoculated at the same time and under the same conditions as those of the first part of the host-range test, except that five kernels of No. 6 Junior wheat were planted in each pot. The twenty-five pots were divided into five series of five pots each. The first series of pots received no fertilizer, while each of the other four series received one of the following treatments:

1. One gram of sodium nitrate per pot, applied by dissolving in 100 cubic centimeters of water which was then poured over the soil.
2. One-half gram of acid potassium phosphate (dibasic) per pot, mixed with the soil.
3. One gram of sulfur per pot, mixed with the soil.
4. Three grams of hydrated lime per pot, mixed with the soil.

After seventeen weeks of growth, no marked differences were found among the different series. Later, the series to which lime had been added was the first on which perithecia were found. This series had more killed plants (15.7 per cent) than the sodium nitrate series (13.6 per cent), the acid potassium phosphate series (5 per cent), or the sulfur series (none), and had less dead plants than the series to which no fertilizer had been added (25 per cent).

After eight months of growth, the plants in all of the series except the one to which sulfur had been added were heavily infected, were badly stunted, and had produced no heads. Even the plants in the sulfur series showed considerable infection, but those in three of the pots had produced several heads. The sulfur apparently produced an acidity of the soil which seemed to have delayed the attack of the fungus or lessened its severity.

From the literature and the preliminary test on the effect of fertilizers, there appears to be a definite relation between the hydrogen-ion concentration in the soil, and the severity of take-all. In order to further test this point, the fungus was grown in culture on an acidity series consisting of potato agars of nineteen different degrees of acidity ranging from pH 3.2 to 9.6, and on bacto-cornmeal agars of ten different degrees of acidity ranging from pH 3 to 9.2. The degree of acidity of the media was determined by the colorimetric method as recommended by Clark.[10]

Cultures of *Ophiobolus cariceti* from three sources, in comparison with one strain of *Fusarium moniliforme* Sheld., were grown on the media in total darkness at room temperature (15° to 20° C.). The average growth of the three strains of *Ophiobolus cariceti*, and the growth of the single strain of *Fusarium moniliforme* at the end of eight days, are shown in figure 4. The results indicate that the take-all fungus requires a condition of alkalinity for optimum growth, and may explain why additions of alkaline substances to the soil have been reported as favoring the fungus while additions of acid-forming substances decrease the amount of infection.

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[10] Clark, W. Mansfield. The determination of hydrogen ions, p. 1–317. 1920.

In order to determine the effect of acidity on the amount of infection, the following test was made in the greenhouse. Sodium hydroxide and sulfuric acid were added in varying amounts to lots of soil so that eighteen degrees of acidity, ranging from pH 2.3 to 8.8, were obtained. The soil comprising each degree of acidity was distributed in five 5-inch pots on February 17, 1922, and five seeds of No. 6 Junior wheat were planted in each pot. In three of the five pots a pure culture of *Ophiobolus cariceti* was added at the time of planting. Within three months after the date of planting, the plants growing in inoculated soils having a pH value from 5.2 to 8.6 were all dead. The plants growing in soils having pH values of 3, 4, and 2.3 were still alive and showed few symptoms of take-all. These plants remained alive until the test was discontinued at the end of nine months. They never grew higher than a few inches, because of the unfavorable soil condition. The plants in the soil having a pH of 8.8 remained alive for about six months, when they all succumbed to the attack of the disease. This experiment seems to show that when sufficient inoculum is present and the plants are grown under greenhouse conditions, no degree of acidity at which plants will normally grow to maturity will allow them to escape being killed by the disease. The plants in the uninoculated pots produced the most heads and grew the tallest at pH values between 7.8 and 8.4, and no heads were produced by plants growing in soil having a pH value as low as 3.4.

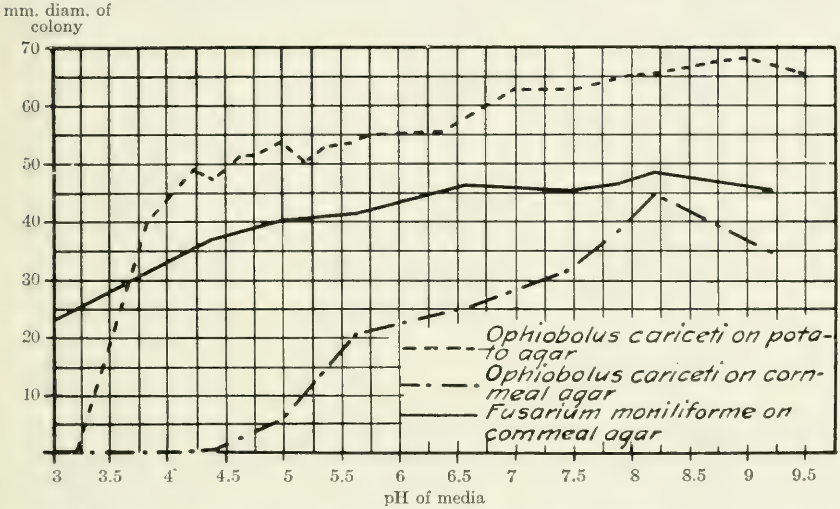

FIGURE 4. RATE OF GROWTH OF OPHIOBOLUS CARICETI AND FUSARIUM MONILIFORME ON AGARS HAVING VARYING DEGREES OF ACIDITY

In order to determine further whether acidity of the soil was an influencing factor on the disease, a test plot at Ithaca was laid out in which certain substances were applied to the soil. There were six series of eleven rod-rows each, which differed from one another only in the substance added. In the first series lime was applied at the rate of 1500 pounds per acre; in the second, sulfuric acid, 300 pounds per acre; in the third, lime, 500 pounds per acre; in the fourth, sulfuric acid, 100 pounds per acre; in the fifth, sulfur, 1500 pounds per acre; and in the sixth, sulfuric acid, 50 pounds per acre. The arrangement of the rod-rows in each series for testing each of the above substances was as follows:

Row 1	No inoculum	No substance added
Row 2	Pure culture of *Ophiobolus cariceti*	No substance added
Row 3	Pure culture of *Ophiobolus cariceti*	No substance added
Row 4	Pure culture of *Ophiobolus cariceti*	No substance added
Row 5	Pure culture of *Ophiobolus cariceti*	No substance added
Row 6	Pure culture of *Ophiobolus cariceti*	Substance added
Row 7	Pure culture of *Ophiobolus cariceti*	Substance added
Row 8	Pure culture of *Ophiobolus cariceti*	Substance added
Row 9	No inoculum	Substance added
Row 10	No inoculum	Substance added
Row 11	No inoculum	Substance added

All rows were planted with No. 6 Junior wheat, and the inoculum and acidifying substances were added on the day of planting. During July, 1922, the rows were harvested by digging up the plants and taking the percentage of diseased plants, the degree of stunting, and the yields. These data, summarized in table 4, show that the addition of lime increased the yield of take-all-free plants, but that it increased the percentage of infected and badly stunted plants to such an extent that the rows yielded less than did those that received an application of acid. This was due to the fact that the healthy plants in the rows growing in the very acid soil yielded only half as much as did those in the very alkaline conditions.

Date of planting

Hori (1901) states that in Japan there is a close relationship between the time of sowing and the severity of take-all, and cites several cases in which barley sown between October 13 and October 27 was much more heavily damaged than was that sown two or three weeks later. In order to get data on the relation of the date of sowing to the amount of disease, two rows each of No. 6 Junior and Forward wheats were sown in infested soil on September 6 and September 29, 1921. The results which are given in table 5, show that later planting decreases the percentage of disease, but at the same time it decreases also the number of heads per plant.

TABLE 4. EFFECT ON THE AMOUNT AND DEGREE OF SEVERITY OF TAKE-ALL, OF ADDING ACID- AND ALKALI-FORMING SUBSTANCES TO THE SOIL

Substances added to soil at planting time	Weight of seed produced by 100 plants* (grams)	Plants infected with *Ophiobolus cariceti*												Plants not diseased			
		Stunted less than $\frac{1}{2}$ normal height				Stunted $\frac{1}{2}$ to $\frac{9}{10}$ normal height				Little or no stunting							
		Per cent of plants	Number of culms per plant	Weight of 10 heads (grams)	Average weight of seed per plant (grams)	Per cent of plants	Number of culms per plant	Weight of 10 heads (grams)	Average weight of seed per plants (grams)	Per cent of plants	Number of culms per plant	Weight of 10 heads (grams)	Average weight of seed per plant (grams)	Per cent of plants	Number of culms per plant	Weight of 10 heads (grams)	Average weight of seed per plant (grams)
1500 pounds of lime per acre	38.88	47.0	0.14	0.065	0.0009	41.0	1.12	2.845	0.319	12.0	2.7	6.4	1.73	0.0	....	......	....
500 pounds of lime per acre	90.57	12.0	0.33	0.35	0.0011	45.0	1.09	2.845	0.312	37.0	1.68	9.54	1.58	6.0	2.6	11.6	3.01
No application	79.46	30.0	0.50	0.30	0.015	33.5	1.20	2.82	0.348	33.5	2.1	8.3	1.77	3.0	2.5	11.4	2.85
50 pounds of sulfuric acid per acre....	72.78	26.0	0.61	0.942	0.0574	34.0	1.40	2.81	0.394	37.0	1.7	8.25	1.40	3.0	2.4	9.916	2.38
100 pounds of sulfuric acid per acre....	127.95	8.5	1.00	1.02	0.102	13.5	1.7	2.902	0.495	63.0	1.8	7.932	1.43	15.0	2.2	9.19	2.02
300 pounds of sulfuric acid per acre....	129.50	4.5	1.00	1.23	0.123	25.0	1.36	3.59	0.489	47.5	1.72	8.78	1.51	23.0	2.5	7.812	1.957
1500 pounds of sulfur per acre........	111.56	0.0	....	......	......	4.0	2.00	2.72	0.54	31.0	1.07	6.34	0.676	65.0	1.87	7.92	1.36

* The figures in this column represent the sums of the weights of 100 plants taken from the respective plots in the proportions indicated by the percentage of plants in each of the four classes, that is, (1) those less than one-half the normal height, (2) those from one-half to nine-tenths of the normal height, (3) those showing little or no stunting, and (4) those not diseased.

TABLE 5. RELATION OF DATE OF SOWING TO AMOUNT OF DISEASE

Date planted	Take-all-infected plants						Plants not infected	
	Plants less than $\frac{1}{2}$ normal height		Plants $\frac{1}{2}$ to $\frac{9}{10}$ normal height		Plants of normal height			
	Per cent of plants	Number of heads per plant	Per cent of plants	Number of heads per plant	Per cent of plants	Number of heads per plant	Per cent of plants	Number of heads per plant
September 6	9.0	0.43	15.0	2.2	70.0	2.5	6.0	2.4
September 29	2.0	0.48	14.8	1.4	70.6	1.1	12.6	1.1

Rate of seeding

In Europe, where take-all is not sharply defined from several other foot rots, Guerrapain and Demolon (1913), Mangin (1914), and Foex (1919 b) all state that increasing the rate of seeding is favorable to the disease. In order to determine this point, test rows of winter wheat were planted in the 1921 plot at Ithaca and inoculated with pure cultures. The results, given in table 6 show that the percentage of badly dwarfed plants increases with the heavier seeding of the land.

TABLE 6. EFFECT OF RATE OF SEEDING ON THE DISEASE

Rate of seeding per rod-row	Per cent of take-all-infected plants			Per cent of plants not infected
	Plants less than $\frac{1}{2}$ normal height	Plants $\frac{1}{2}$ to $\frac{9}{10}$ normal height	Plants of normal height	
Light, $7\frac{1}{2}$ grams	23.2	30.3	46.5	0.0
Normal, 15 grams	30.0	33.5	33.5	3.0
Heavy, 30 grams	38.0	34.0	28.0	0.0

CONTROL

EXCLUSION

A quarantine to prohibit the importation of possible harborers of the pathogene was attempted in the United States in 1919 (Ausley, 1919). Present evidence, such as the general distribution of take-all in the State of New York and its known occurrence in Oregon, Arkansas, Kansas, Indiana, Tennessee, North Carolina, and California, indicates that it has been present in this country for many years but has only recently been recognized. Since the pathogene apparently was generally present in the United States previous to the laying of the quarantine, this attempt at exclusion cannot be expected to be of any importance in the control of the disease.

ERADICATION

Investigators agree that when the take-all pathogene has once become established in an area the most practical method of control is by eradication. The methods to be employed involve the killing of the ascospores and the starving-out of the fungus.

Cultivation has for its primary object the destruction of grass and cereal harborers of the pathogene, and to accomplish this, the ground, as Dawkins (1914) states, must be kept absolutely free of any grass on which the fungus can thrive. This can best be done, according to Sutton (1911), by cultivation soon after the wheat harvest and subsequent working of the land after each rain. Other investigators, among them Gray (1914), recommend one or two whole years of fallowing. The fallowing of the ground for one or more years, as suggested for the Australian conditions, cannot be followed everywhere.

In New York the usual rotation practice makes it impossible to plow the land after the wheat harvest. Rotation must be depended upon here to starve out the fungus.

There is a considerable difference of opinion as to the required length of this rotation. If susceptible grasses, barley, and rye are not present in the rotation, the length is given by Sutton (1911) as two years, by McAlpine (1904) and Gray (1914) as three years, and by Richardson (1911) and others as from four to six years. The importance of rotation as a control measure may be inferred from the fact that during the 1921 and 1922 surveys in New York, it was observed that in nearly every field in which more than five per cent of the plants were killed by take-all, two or more successive crops of wheat had been planted. A rotation, to be effective, should have wheat not oftener than every four or five years, since the fungus can live in the soil for five months, and on the stubble as spores for from nine to ten months; and if *Agropyron repens* or other wild hosts are present, several years of cropping may be required to kill out the grass and with it the fungus.

The stubble of diseased wheat being the principal harborer of the take-all fungus, some means of disinfection, such as burning the stubble, should be applied to destroy this source of inoculum. Under Australian conditions McAlpine (1904), Richardson (1911), and Brittlebank (1920) advocate this means of disinfecting the field, but Sutton (1911) says that a stubble burn is of doubtful value since it does not destroy the underground parts on which perithecia are produced. Under New York conditions the burning of stubble would kill the clover seeding which is planted with wheat, and would thus be of doubtful value. Straw, which would be likely to contain culms broken or cut off low enough to make them carriers of the organism, should be disposed of in such a way that none of the stubble or straw is returned to the land in manure or in other ways for at least three years preceding the planting of wheat. Observations in 1921 showed that wheat straw from diseased fields, when applied in manure during the fall and winter of 1920–21, acted as a direct carrier of the fungus.

PROTECTION

The first measure by which protection is to be obtained is by modifying moisture relations. Humidity of the soil has been reported as being favorable to the disease (Robinson, 1907, and Mangin, 1914), and the present investigations seem to confirm this theory. Low, wet soil should therefore be drained before the wheat is planted.

The second measure of portection is by modification of temperature relations. From the evidence presented under *Ecology*, it appears that high temperatures in the fall increase the amount of seedling infection. The planting of winter wheat as late as possible will reduce the amount of infection and the resulting amount of diseased and badly dwarfed plants at harvest time.

The third measure of protection is by modifying the chemical reaction of the soil. Brittlebank (1920) found that an alkaline soil produced more take-all than one which was non-alkaline. The present investigations show that the addition of lime increases, and the addition of acid or acid-forming substances decreases, the percentage of badly diseased plants. A study of the germination of ascospores and the rate of growth of the organism at different degrees of acidity indicated that acid added to the soil acts as a barrier between the pathogene and the susceptible roots of the host plants, in some way, probably by inhibiting the germination of the ascospores and the growth of the fungus. It was observed also that the yield of uninfected plants was reduced as the acidity increased below pH 7. The advisability of using acid on the soil to control take-all would therefore be dependent on such factors as the severity of the disease in any one locality and the degree of tolerance to acid conditions of the other crops in the rotation.

The results seem to indicate that where wheat, barley, rye, and potatoes are the only crops in the rotation, and where the disease is severe, the application of a moderate amount of an acid-forming substance would be a profitable control measure. In New York certain crops such as clover, which is intolerant to acid conditions, are often grown in the rotation. It therefore seems unlikely that the addition of acid would be practicable here. Nevertheless, from the point of view of take-all control, the practice of adding lime to the soil before planting wheat should be discontinued.

IMMUNIZATION

From the evidence presented under *Varietal Susceptibility*, it is probable that such varieties of wheat as Forward and Dawson will be found resistant to the disease under field conditions and may be recommended for planting in areas where take-all is most severe.

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THE GRAY BULB-ROT OF TULIPS CAUSED BY RHIZOCTONIA TULIPARUM (KLEBH.) N. COMB.

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THE GRAY BULB-ROT OF TULIPS CAUSED BY RHIZOCTONIA TULIPARUM (KLEBH.) N. COMB.

H. H. Whetzel and John M. Arthur[1]

The gray bulb-rot of tulips, long known in Holland and Germany, apparently has not until recently (Whetzel and Arthur, 1924) been reported from North America. In April, 1922, Dr. William Crocker, Director of the Boyce Thompson Institute for Plant Research at Yonkers, New York, sent some diseased bulbs from a bed in a near-by garden to the Department of Plant Pathology at Cornell University for diagnosis. Dr. L. M. Massey obtained from these bulbs pure cultures of a sclerotial fungus. These cultures were turned over to the senior author the following June. Their marked similarity to cultures of *Sclerotium tuliparum* Klebahn which he had obtained from Holland some years ago and was still carrying along, seemed to warrant a further investigation of the disease. A cooperative undertaking for this purpose was at once arranged between the two institutions, the results of which are here set forth.

THE DISEASE

HISTORY[2]

This disease was known in Holland at least as early as 1884, when it came to the attention of Wakker (1885:22), who was then engaged in an investigation of bulb diseases for the Dutch Bulb-Growers' Association.

Wakker appears to have been the first to describe the disease, which he designated merely as the "tulpenziekte" (tulip disease). His observations on the symptoms of the disease and the character of the pathogene are extraordinarily clear and accurate.

Ten years later Ritzema Bos (1894:229) began an investigation of the disease, which he found very destructive in certain sections of Holland at that time. Although he refers to the earlier work of Wakker, he appears to regard it as of little importance. In his later report (1903a:178 [p. 23 Centbl.]) he describes symptoms which are undoubtedly those of the gray bulb-rot, but confuses this disease with the Botrytis blight, which he also had before him (Hopkins, 1921:316–317). Ritzema Bos apparently assumes

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[1] Biochemist of the Boyce Thompson Institute for Plant Research.

[2] In view of the fact that no previous paper on this disease has appeared in English, and since several of the most important articles cited in this discussion are not readily accessible to American pathologists, a rather full review of the previous literature on the bulb-rot is here presented.

(1903 a: 190) that Wakker also had only the Botrytis blight under consideration when he described his "tulpenziekte."

Because of the failure of tulips to come up in the spring in soil-infested areas, the disease is called by the Dutch growers "wegblijven" (away remaining) and the contaminated areas "kwade plekken" (bad spots) or "kwade grond" (bad ground). From the fact that Wakker does not use these terms in his early description of the disease, Ritzema Bos (1903 a: 191) concludes that the disease is new to Dutch bulb-growers in spite of the assurance of growers in Noordwijk that it had been known to them for more than twenty years.

Klebahn (1904: 18) observed the disease in the spring of 1903 in the botanical gardens at Hamburg, Germany, and began a series of investigations culminating in three papers (1905, 1906, and 1907), the most important contributions on the subject to date. In his early work (1904) he too confused the gray bulb-rot with the Botrytis blight, but he soon discovered that he was working with two distinct diseases. In his later papers he clearly distinguished the gray bulb-rot from the Botrytis blight, giving it the name *sclerotium disease* (1905: 1–18).

As none of the names thus far applied to the disease appear to be entirely satisfactory, the term *gray bulb-rot* is proposed as indicating more definitely its character.

RANGE AND IMPORTANCE

The gray bulb-rot, which seems to have first appeared in Holland in the early eighties, has gradually become widespread and destructive in certain sections of the bulb-growing districts there. That it was of relatively little importance in 1884 seems evident from the meager consideration given it by Wakker (1885), who, however, reports it as sometimes destroying most of the tulips in a bed. Ritzema Bos (1894: 229) reports what was undoubtedly this disease as having been very destructive in scattered localities in the bulb districts during the seasons of 1892 and 1893, and again (1895: 348) in 1894. In his report ten years later on the investigation begun at that time, he intimates (1903 a: 177–178) that the disease had in the meantime become much more general and serious in the bulb districts, and that it was even then becoming a factor in the export trade in bulbs to adjacent countries, because of losses to gardeners in Germany and elsewhere who purchase the bulbs for forcing and outdoor plantings. Since, however, he constantly confused the bulb-rot with the Botrytis blight, one cannot estimate from his reports the relative importance of the two diseases.

Klebahn (1905: 13–14) concluded from his early inoculation experiments that the bulb-rot was far more dangerous and serious than the Botrytis blight, but from observations made shortly afterwards on a visit to the bulb-growing districts of Holland he observed (1907: 3) that the Botrytis blight was not so harmless as he had been led to believe, that it was much more widespread than the bulb-rot, and that it caused the growers much trouble. There is, however, little doubt that the bulb-rot is a serious disease in regions where it is well established (Klebahn, 1907:12–13).

The disease has been reported from Switzerland (Müller-Thurgau, 1908:750, and Lendner, 1911), and also by Jaczewski from Russia (Diakonoff, 1913:281). Although no reference to the disease in England has been found in the literature,[3] it undoubtedly occurs there; to what extent, however, is not known. The senior author received a culture of the pathogene isolated by Miss Wakefield from tulip bulbs in Kew Gardens in 1922. It is remarkable that it has not previously appeared in America, or, if it has, that it has not sooner attracted attention. With the growing restrictions on the importation of Dutch bulbs, and the possibility that Americans must eventually depend more and more on home-grown stock, the appearance of the disease in this country assumes a more than scientific interest.

The bulb-rot is of greatest economic importance in those districts where bulbs are grown for sale and export, as in Holland. Even there it appears to be confined to certain districts (Klebahn, 1907:12–13). Since the pathogene appears to be transported but rarely on bulbs in commerce and is apparently not disseminated by spores, it is only occasionally destructive in tulip beds grown for blossoms, and its ravages are therefore largely confined to the beds that became contaminated by the original importation. Klebahn (1904:21–22), reports, as a result of a survey of the tulip plantings about Hamburg, that the disease is rarely met with, and then only in an occasional planting of bulbs for flowers. The fields of the one grower near Hamburg who produced bulbs for the trade were free from the disease. Klebahn reports a few isolated cases of the disease in tulips imported from Holland into other parts of Germany (1907:4).

The gray rot appears, therefore, to be a disease of relatively little danger to tulip-growing in America, where flower production is at present the chief object, even though the bulbs continue to be imported from bulb-growing countries such as Holland, where the pathogene is well established. Should a tulip-bulb-growing industry develop in the United States, the infrequent transportation of the pathogene on bulbs, the slowness with which it spreads from bed to bed, and the ease with which contaminated soil may be disinfected, does not warrant any particular concern as to the continued importation of Dutch bulbs into this country.

SYMPTOMATOLOGY

The first evidences of the disease are the bare spots in the tulip beds in the spring, where the plants fail to come up. Nearly all bulbs in the soil-contaminated area are usually so injured that they fail to grow. When affected bulbs do send up leaves, their growth is greatly retarded. They soon die and wither away, usually before they can expand, because the fungus gradually rots them off underground. Initial infection evidently occurs in the fall and early winter shortly after the bulbs are put out into the beds, or early in the spring. Observations by the authors on the symptoms of the disease check fully with the description and illustrations of them given by Klebahn (1905, figures 4–5, and 1906, figures 53–55).

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[3] The reference to this disease by Bewley in his book *Diseases of Greenhouse Plants* (1923:71) is an error. He has mistaken a Botrytis disease (not caused by *B. tulipae*) for the sclerotium disease, as indicated by cultures of his fungus which he sent to the senior author. His citation of the article by Ramsbottom is also an error, for that deals only with the nematode of hyacinths.

When diseased bulbs are dug up, they are found to be more or less rotted, the infection beginning usually at the tip, or nose, of the bulb. The roots are in most cases abundant and healthy (Plate I). The leaves and the flower shoot, if they have started, are rotted, and the adjacent bulb scales within are more or less involved. The rot is a dry one, the tissue for a time remaining rather firm (Plate II). The healthy white tissue is turned to a grayish or a reddish gray color, a very distinctive symptom of this disease.

On digging up the diseased bulbs, one is at once struck by the fact that the soil clings tenaciously to the exterior of the rotted parts. This is evidently due to the external mycelium, which binds the soil particles together and holds them to the rotted bulb scales. Embedded in the soil about the bulb are numerous, more or less globose, dark brown bodies, the sclerotia of the pathogene. These, when dry, become almost black. They vary from 1 to 9 millimeters in diameter. They are often present also in large numbers on the surface of the rotted scales and neck of the bulb under the adhering soil (Plate I). The sclerotia are but loosely attached to the surface of the diseased tissues, and come away easily with the removal of the soil. Some are occasionally to be found within the rotted bulb between the decayed scales. There is an abundance of grayish white mycelium on the outer surface of the bulb covering the diseased parts (Plate I). Within the bulbs this mycelium frequently forms a felty layer between the diseased scales (Plate II).

ETIOLOGY

Identity of the pathogene

The cause of the gray bulb-rot is a fungus named by Klebahn (1905:13) *Sclerotium tuliparum*. Wakker (1885) appears to have been the first investigator to discover and describe this pathogene, though he did not give it a name. He, however, pointed out that it differs fundamentally in many features of its structure from *Peziza* (*Sclerotinia*) *bulborum*, the cause of the black rot of hyacinths, which he at that time had under investigation. Ritzema Bos (1903 a:182), who next investigated the disease, confused the causal organism with *Botrytis tulipae* (Lib.) Hopkins.[4] He mentions the earlier work of Wakker, but attributes the disease there described to *Botrytis tulipae* also. It is evident from his description of the symptoms (1903a:178-181) that he was dealing with bulbs affected with *Sclerotium tuliparum*, as well as some attacked by *Botrytis tulipae*. This conclusion is further supported by his observations (1903a:186-187) on the two types of sclerotia and mycelia which he found in the diseased bulbs.

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[4] Hopkins (1921:328–331) has shown *Botrytis parasitica* Cav. to be identical with *Sclerotium tulipae* Libert, and has made the combination *Botrytis tulipae* (Lib.) Hopkins.

Klebahn (1904:19-21), investigating the diseases of tulips in the gardens in Hamburg in the spring of 1903, described the symptoms of the gray-rot disease and, following the lead of Ritzema Bos, likewise attributed it to *Botrytis tulipae*, with which he made infection experiments. The large sclerotia which he found in the soil and about the crowns of rotting tulips, and which failed to develop in his cultures of *Botrytis tulipae*, led him to doubt their identity with the small sclerotia of this species as is shown by the following: " Diese Umstände zeigen allerdings zunächst nur, dass zu der *Botrytis* überhaupt Sklerotien gehören, aber nicht unbedingt, dass die an den krankeu [*sic*] Zwiebeln aufgefundenen grossen Sklerotien (Fig. 3) mit der auf den Blättern vorkommenden Botrytis in Zusammenhang stehen." (Klebahn, 1904, figures 1 and 3.)

Continuing his investigations, Klebahn soon (1905:14-17) came to the conclusion that the large sclerotial fungus with which he was working is distinct from *Botrytis tulipae* as well as from *Sclerotinia bulborum*, since he was unable to obtain any conidial or sexual fruiting structures. His later investigations confirmed this conclusion but disclosed nothing further as to the taxonomic relationships of the fungus. He named it *Sclerotium tuliparum* (1905:13), apparently being unaware that this name had already been applied by Schlechtendal (Bouché, 1831:223) to the fungus now known as *Botrytis tulipae*.

The fungus associated with the rotting tulip bulbs in the beds near the Boyce Thompson Institute corresponds in all respects, both in nature and in pure culture, with the descriptions and illustrations given by Klebahn. Moreover, comparisons of pure cultures with cultures of the fungus received from Holland[5] and England[6] show only such slight cultural differences (Plate III) as are to be attributed to physiological variations of individual strains. There appears, therefore, to be no doubt that we are here dealing with the pathogene now commonly known to European workers as *Sclerotium tuliparum* Klebahn.

Taxonomic relationships

In the course of these investigations, certain distinctive characters in the morphology of the pathogene have been observed which seem to afford a more satisfactory taxonomic placing of the fungus than in the form genus Sclerotium, where it was left by Klebahn.

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[5] Three strains were isolated from sclerotia on bits of tulip bulbs sent to the senior author by Ritzema Bos in 1915. Another culture from Holland was obtained from the pure-culture laboratory of Dr. Westerdijk at Baarn in 1924. All of these were identified by the senders as *Sclerotium tuliparum* Klebh.

[6] The culture from England isolated by Miss Wakefield from tulips at Kew Gardens was sent to the authors from the Lister Institute, Chelsea Gardens, London; their number, 1497.

In pure cultures on potato agar, the fungus forms an appressed white mycelium which is inclined to a radiate rhizomorphic formation (Plate III, A). The medium is soon discolored, often taking on a distinctly reddish tinge which deepens with age. The sclerotia first appear as irregular, white, cottony masses on the surface of the culture, in a broad zone at some distance from the center of the colony (Plate III, A, B, and C). They soon turn to a pale yellowish buff, deepening to a dark reddish brown, and eventually become almost black when dry. The sclerotia vary greatly in size and form. They are, in general, globose to loaf-shaped bodies, 1 to 9 millimeters in diameter, and in many cases several are agglomerated into a large, irregular mass. The surface of the sclerotium is dull, fibrillose, and irregular, as contrasted with the smooth, naked, polished surface of the sclerotia of Botrytis or Sclerotinia.

A cross section through a sclerotium shows the interior to be light yellowish brown in color, but of a much lighter tint than the outer narrow zone forming the rind. This colored medulla is in striking contrast to the white medulla of the sclerotia of *Sclerotinia bulborum* or *S. sclerotiorum* (Plate IV, A and D), to which these sclerotia bear a superficial resemblance. A microscopic examination of thin sections of the sclerotia show further marked differences, which Wakker (1885:23) first recognized and pointed out, but which Klebahn appears to have overlooked. Instead of the narrow, thick-walled, interwoven hyphae which constitute the medulla of the sclerotia of the Sclerotinias (Plate IV, D), one finds large, irregularly globose, thin-walled cells forming a typical pseudoparenchyma (Plate IV, A). Instead of the distinctly differentiated rind of thick-walled, black cells forming the rind of the sclerotia of the Sclerotinias, there is here a pseudorind differing in structure from the medulla only in that the cells are generally more elongated and the walls are darker in color (Plate V, B). As the sclerotium matures and dries, the cells of the pseudorind collapse (Plate V, C). The transition from medulla to rind is gradual, and is marked only by an increasingly deeper tinting of the cell walls. Covering the young sclerotia and arising from the cells on the outside is a loose, wefty covering of septate hyphae, the cells of which are sometimes slightly barrel-shaped and are branched in the manner characteristic of Rhizoctonias. This fibrillose mycelial coating is very abundant in the early stages of the development of the sclerotium (Plate V, A), but collapses and disappears more or less as the sclerotium matures. The walls of this mycelium also become reddish brown with age, but are of a lighter tint than the cells of the pseudorind.

A comparison of the sclerotial structure of *S. tuliparum* with that of *Corticium vagum* (*Rhizoctonia solani*) and *Corticium stevensii* indicates at once its taxonomic relationships. In the early stages of development of the sclerotia, their fibrillose coating (Plate V, A) and the color changes through which they pass remind one strongly of the sclerotia of both *C. vagum* and *C. stevensii*. They differ from these species, however, in several distinct respects. They are compact and definite in form like the sclerotia of *C. stevensii*, instead of diffuse and crust-like as are the sclerotia of *C. vagum*, though some other species of Rhizoctonia produce very definite, more or less globose, sclerotia (Matz, 1921:15). The internal structure is likewise different in certain respects. The cells of the medulla are more globose and compact, showing little of the branched hyphal character of the cells of the medulla of *C. vagum* and *C. stevensii* (Plate IV, B and C). The large intercellular spaces so evident in the medulla of *C. vagum* are almost entirely wanting. The cell walls are much less darkly colored, and there is a distinct pseudorind which is entirely wanting in *C. vagum*. If the structure of the sclerotium of *S. tuliparum* is compared with that of *C. stevensii*, a somewhat more marked similarity is found. The description of the structure of the sclerotium of *C. stevensii* as given by Stevens and Hall (1909:53) does not correspond exactly with the findings of the present authors in an examination of specimens of the fungus sent to them from North Carolina by F. A. Wolf. Stevens and Hall say: "They are entirely devoid of any special epidermal structure, false epidermis, or rind, the peripheral structure being identical with that of the interior." The present authors find that the sections of *C. stevensii* show distinct evidences of a pseudorind (Plate IV, B), due entirely, however, to the collapse of the peripheral cells and not to a deeper coloration of the walls of these cells. The cell walls of the sclerotial hyphae of *C. stevensii* are much darker than those of *S. tuliparum* and uniformly tinted throughout. It is in the collapsed character of the pseudorind that the two species show a similarity. Stevens' drawing (Stevens and Hall, 1909:53, figure 6) gives the impression of thick walls in the cells of the medulla. The authors found that the sections of *C. stevensii* show the walls to be thin, like those in *C. vagum* and *S. tuliparum*. The cells of the medulla are more elongated (often hyphal) than those of *S. tuliparum*, while the distinct intercellular spaces so characteristic of the medulla of the sclerotia of *C. vagum* are much less pronounced. On the whole, the structure of the sclerotium of *S. tuliparum* approaches more nearly that of the sclerotium of *C. stevensii* than it does that of *C. vagum*, though the last two are more alike.

Furthermore, the characters of its mycelium (figures 1, 2, and 3), and its behavior in culture (Plate III), confirm the writers' opinion that *S. tuliparum* is a Rhizoctonia. The branching of the mycelium and the barrel-shaped cells, especially those of the loose hyphae covering the sclerotia, are typically those of a Rhizoctonia. The reddish coloration of the media by the growing mycelium is also a characteristic of many Rhizoctonias.

No basidiomycetous stage has been discovered thus far, but the marked similarity of the mycelium and the sclerotia to these structures in Rhizoctonia species whose sexual stages are known to be Corticiums (in the sense of Burt, 1918) makes it all but certain that, when found, the perfect stage of *S. tuliparum* will prove to be a Corticium.

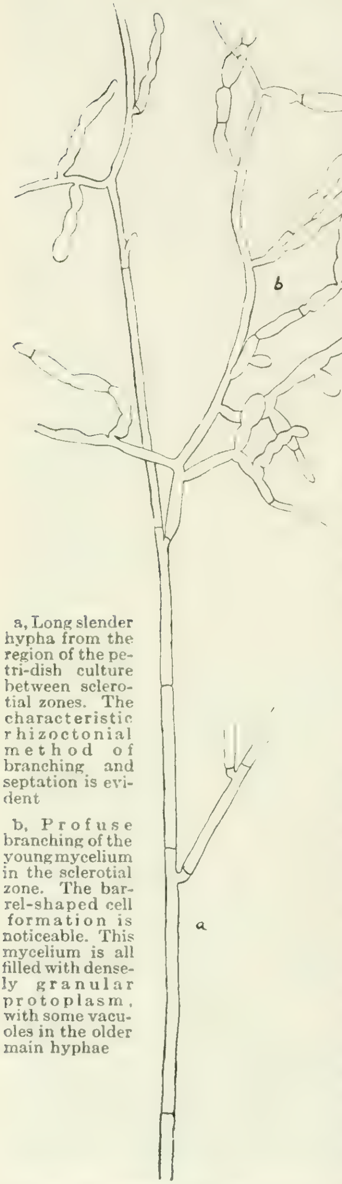
Both Wakker (1885:25) and Klebahn (1905:9) attempted to obtain a fruiting form of the fungus by overwintering the sclerotia in sand or soil. Neither succeeded. Klebahn at least appears to have expected from these sclerotia the production of apothecia.

The writers' investigations seem to warrant the transfer of the fungus to the genus Rhizoctonia. Although Schlechtendal's use of the name *Sclerotium tuliparum* for *Botrytis tulipae* antedates that of Klebahn, it appears legitimate to designate this pathogene *Rhizoctonia tuliparum* (Klebh.) n. comb.

Pathogenicity

Wakker (1885:24–25) concluded from field observations that this fungus was the cause of the rotting of tulip bulbs. He observed also a fungus producing the same sort of sclerotia associated with a rot of hyacinths and a rot of *Dicentra spectabilis.* He made no inoculation experiments with this fungus on any of the three suscepts upon which he observed its occurrence.

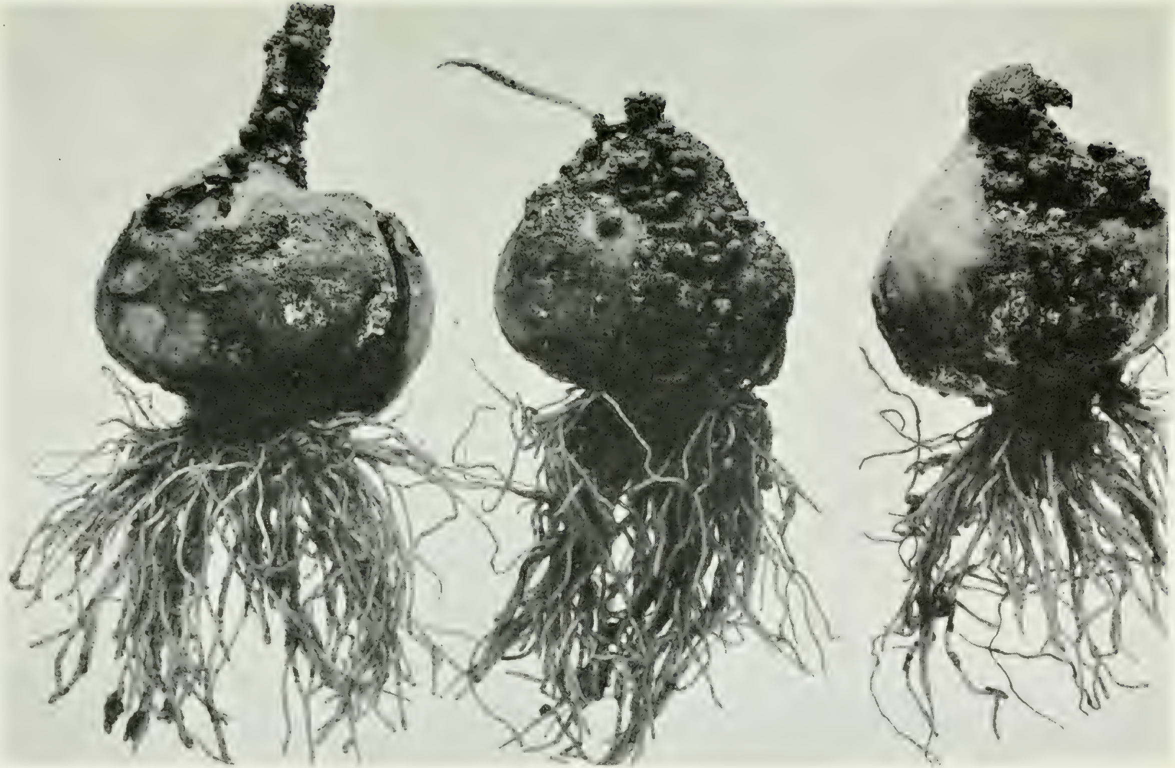
The pathogenicity of this fungus on tulips, however, has been fully established through the careful inoculation experiments of Klebahn (1905 and 1907). He (1907:28–29) demonstrated that it may attack also *Iris hispanica*, hyacinths, *Fritillaria imperiales*, yellow narcissus, and *Scilla sibirica*, but on these suscepts, except for the first, relatively little injury and poor sclerotial formation occur. Klebahn observed that the iris is quite as severely injured as the tulip (1906: 564). He was unable to obtain infection on



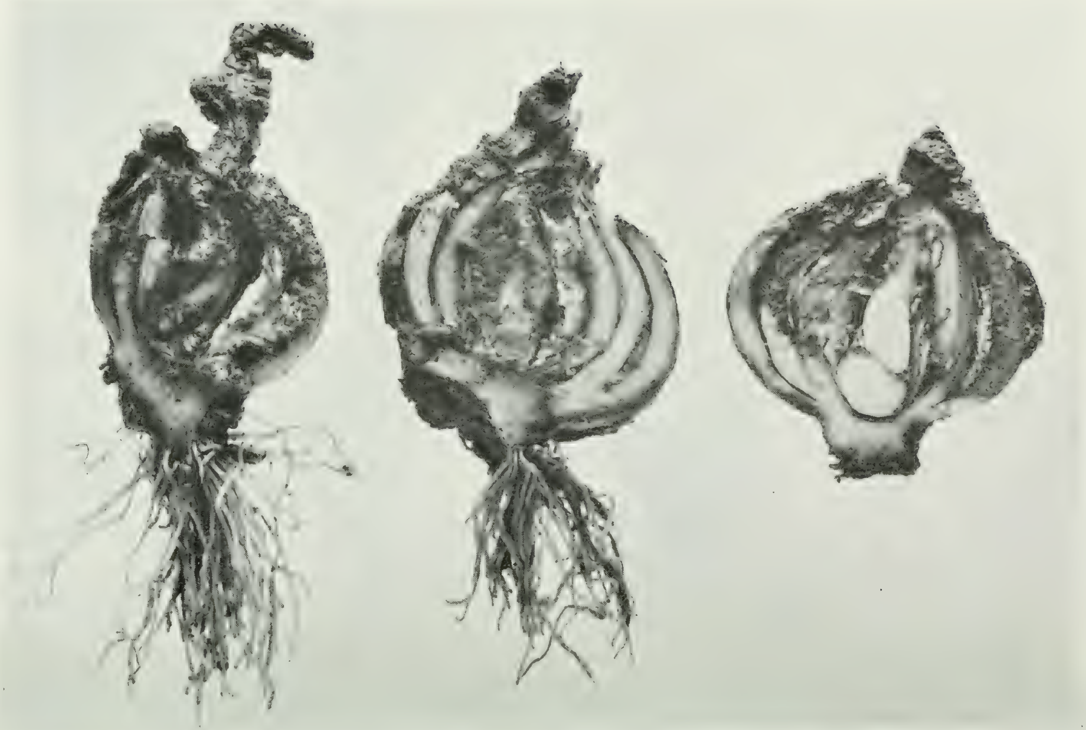
a, Long slender hypha from the region of the petri-dish culture between sclerotial zones. The characteristic rhizoctonial method of branching and septation is evident

b, Profuse branching of the young mycelium in the sclerotial zone. The barrel-shaped cell formation is noticeable. This mycelium is all filled with densely granular protoplasm, with some vacuoles in the older main hyphae

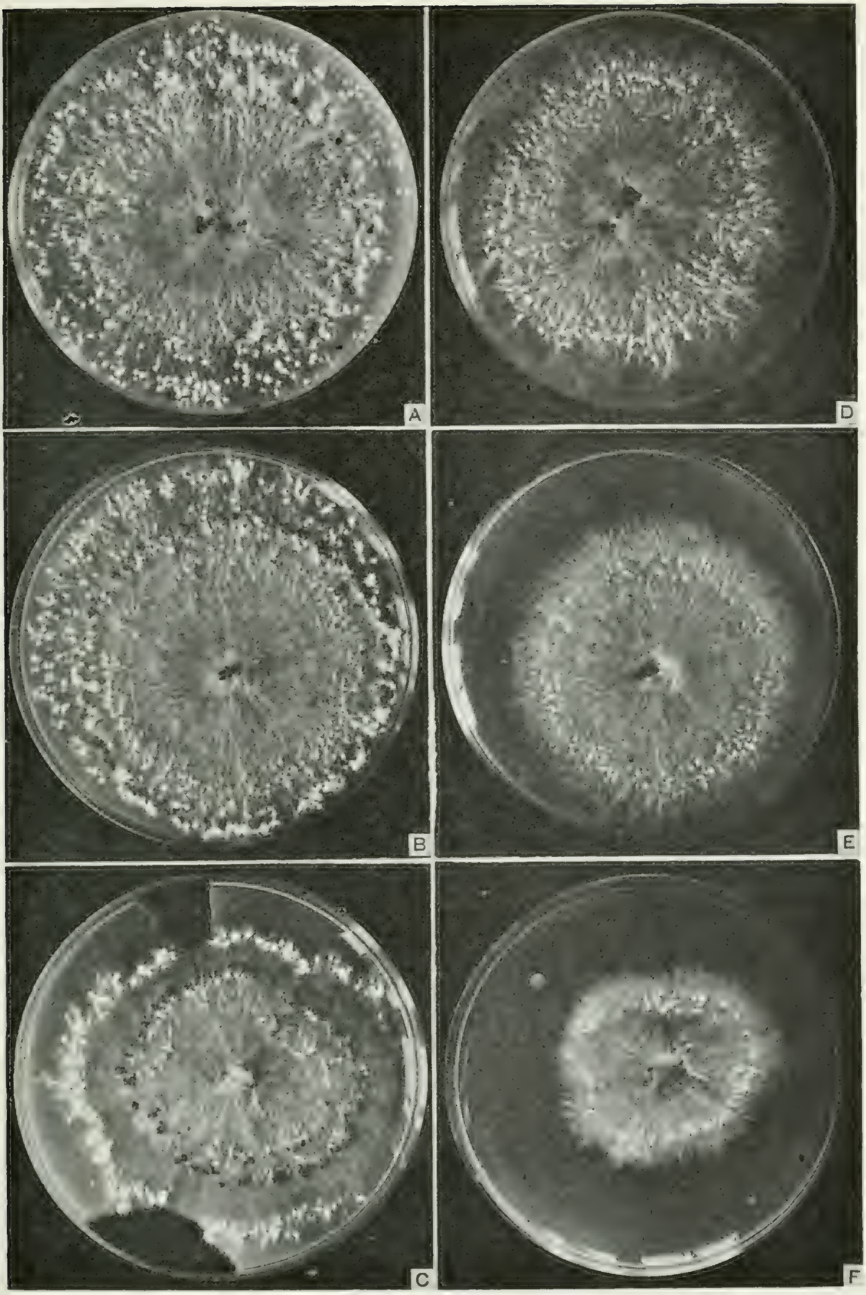
Figure 1. FORM AND SEPTATION OF THE MYCELIUM OF R. TULIPARUM FROM POTATO AGAR



DISEASED BULBS WASHED FREE OF SOIL, SHOWING SCLEROTIA AND EXTERNAL MYCELIUM



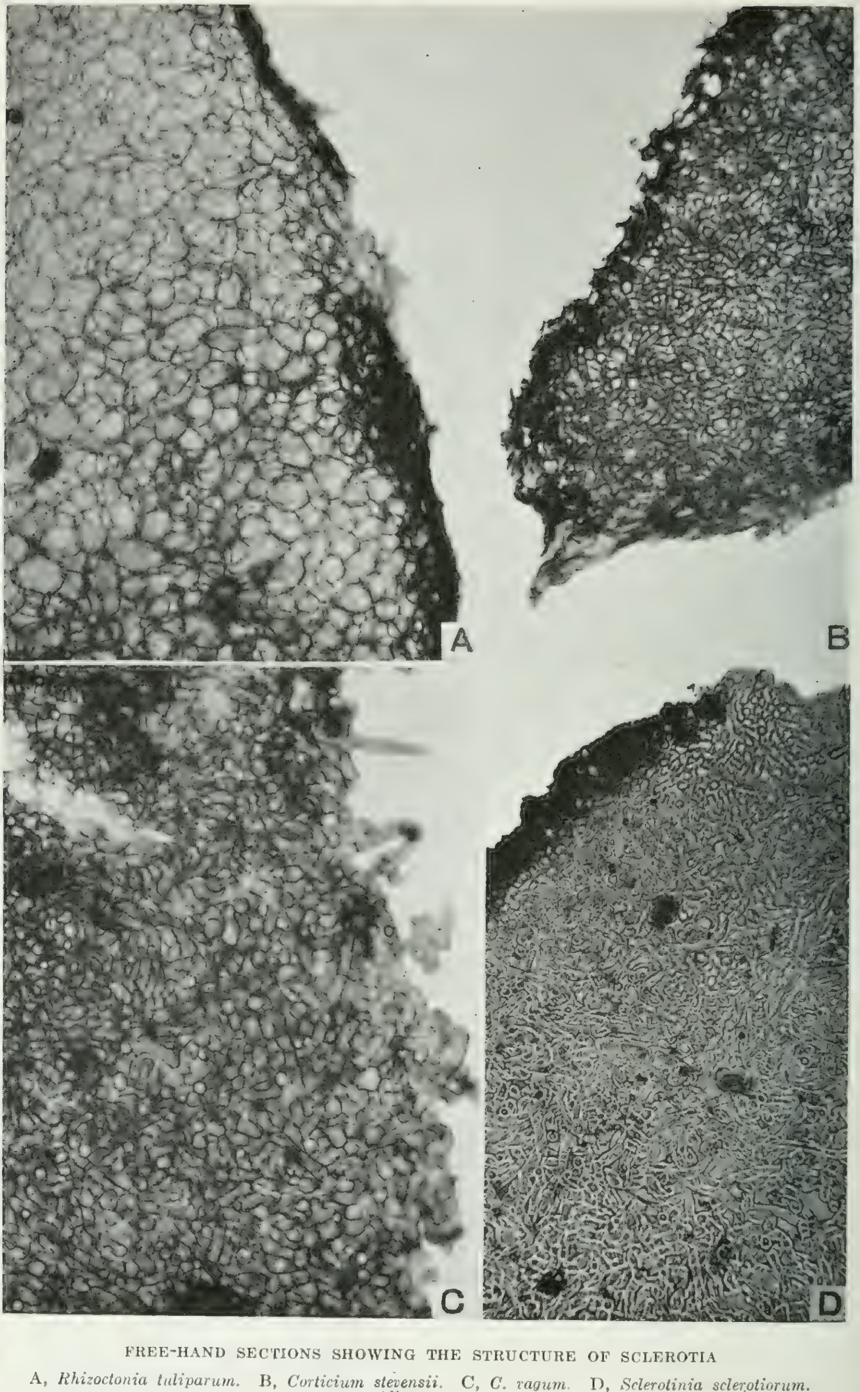
DISEASED BULBS CUT OPEN, SHOWING ROTTED SCALES WITH MYCELIAL FELTS BETWEEN



CULTURES OF THREE STRAINS OF R. TULIPARUM ON POTATO AGAR AT ROOM TEMPERATURE

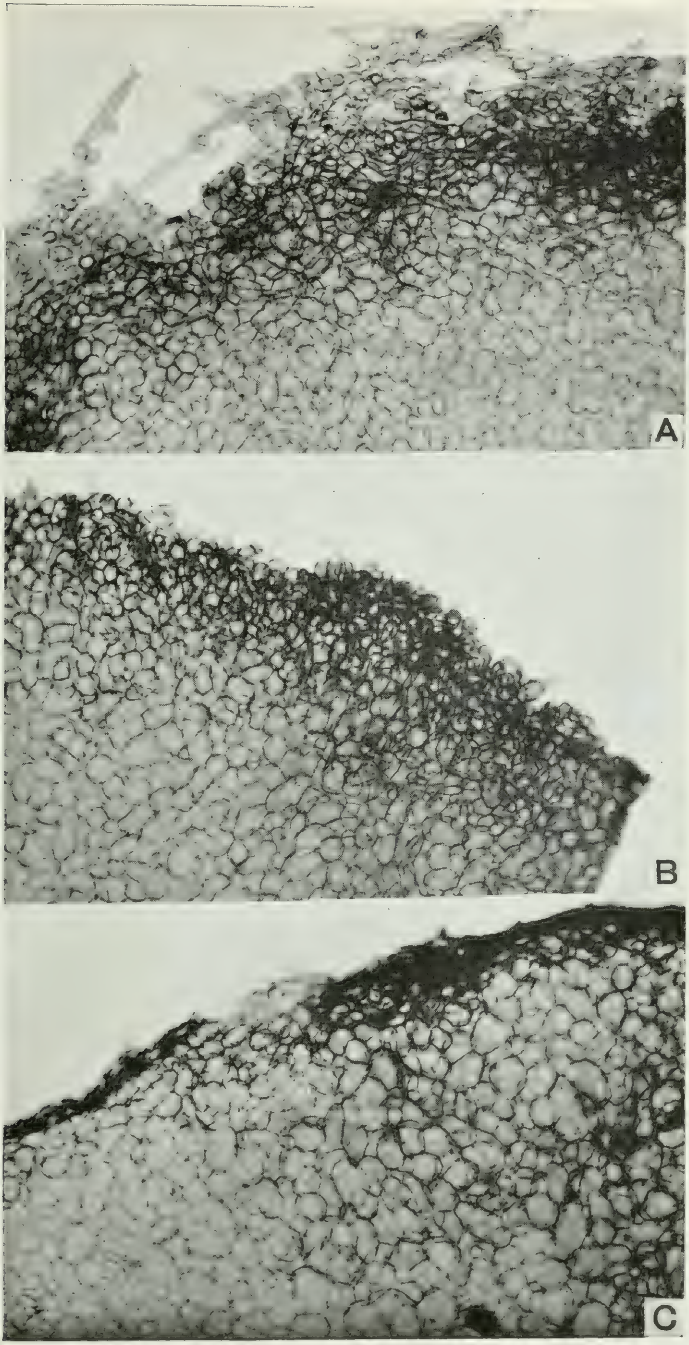
The three plates on the right show growth after ten days, the three on the left the same plates after twenty-three days

A and D, strain (S 195) from Kew Gardens, England. B and E, strain (S 145) from Yonkers, New York. C and F, strain (B xxxv) from Holland. The distinctly slower growth of the Holland strain may be due to the fact that it had been in culture since 1915, while the other two strains were isolated in 1922



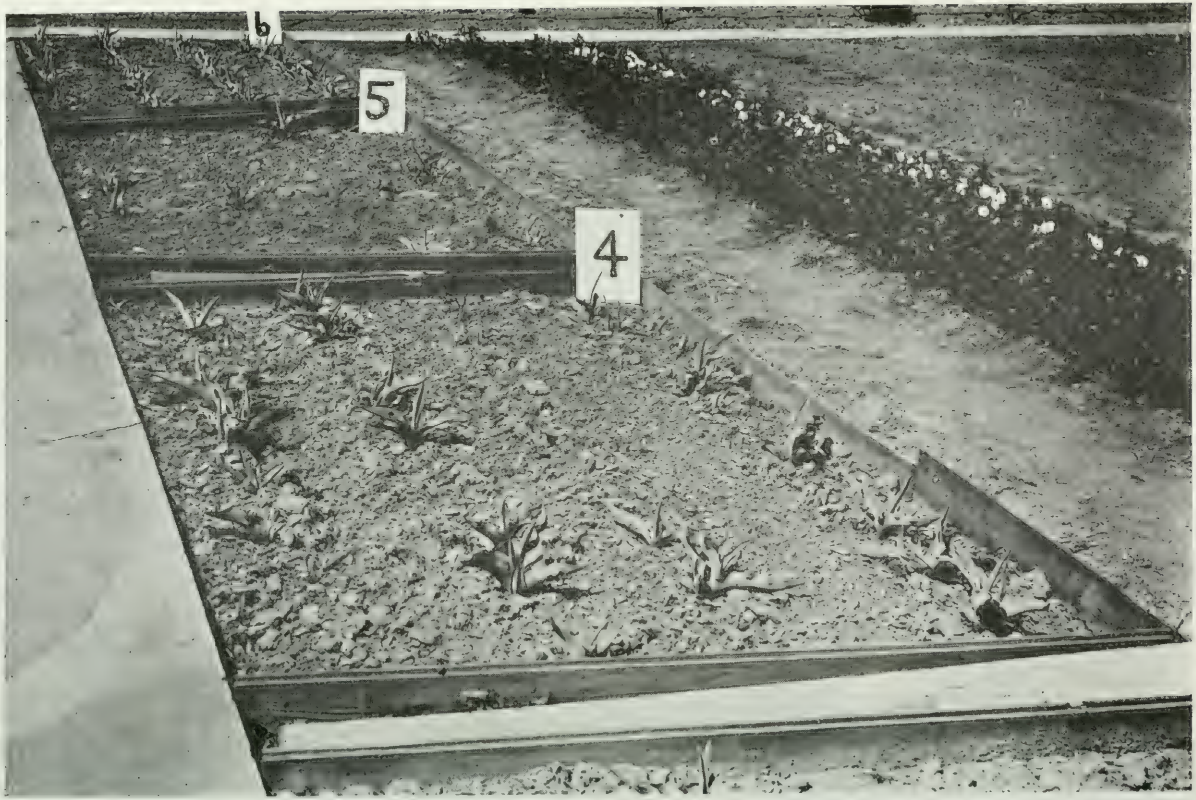
FREE-HAND SECTIONS SHOWING THE STRUCTURE OF SCLEROTIA

A, *Rhizoctonia tuliparum.* B, *Corticium stevensii.* C, *C. vagum.* D, *Sclerotinia sclerotiorum.*
All × 145



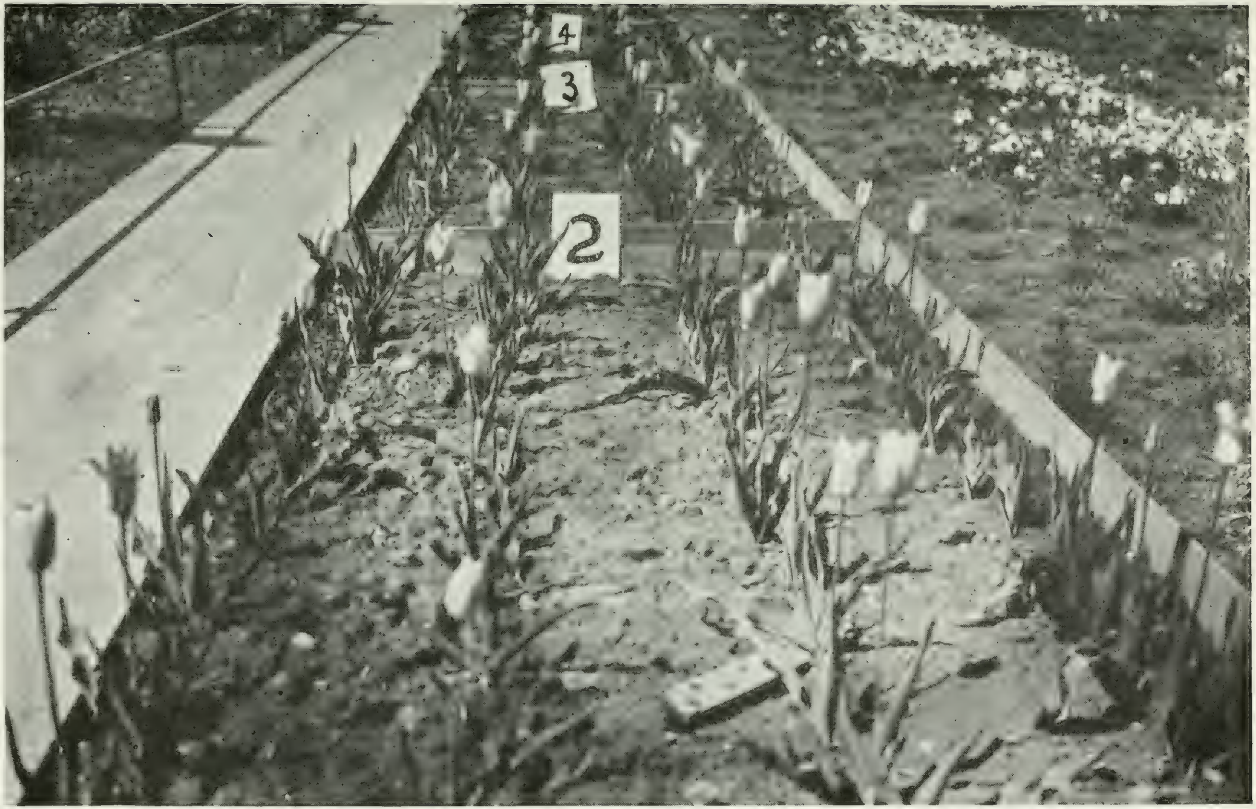
FREE-HAND SECTIONS OF SCLEROTIA OF R. TULIPARUM

A, Young stage of sclerotium from pure culture, showing the external mycelium and the deep coloration of cell walls in the region where the pseudorind is forming. B, Mature sclerotium from pure culture, showing elongated outer cells and deeper-colored walls of the pseudorind; the external mycelial weft is largely torn away. C, Mature sclerotium from bulbs in soil; the cells of the pseudorind have collapsed. All × 145

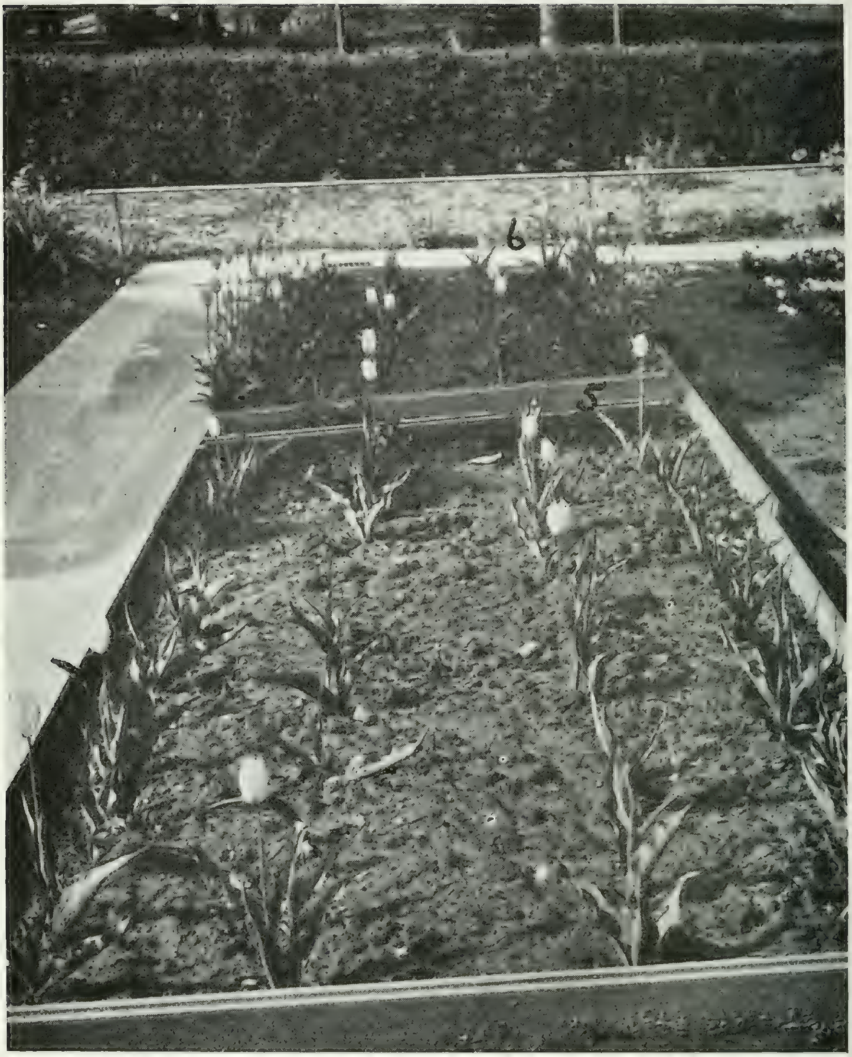


THREE OF THE SIX PLOTS OF THE 1922–1923 CONTROL EXPERIMENTS

Photograph taken in the spring of 1923, shortly after the tulips were well up. Plot 4 treated with 7½ pounds of formalin; plot 5 untreated (check); plot 6 treated with 10 pounds of formalin



THREE STEAM-STERILIZED PLOTS OF THE 1923–1924 CONTROL EXPERIMENTS
Photograph taken when the plants were in full bloom. The almost perfect stand is noteworthy



TWO OF THE PLOTS OF THE 1923–1924 CONTROL EXPERIMENTS

Photograph taken in the spring of 1924 when the plants were in full bloom. Plot 5 treated with 15 pounds of formalin solution; plot 6 untreated (check). The two crowded outer rows of disease-resistant plants from disease-free bulbs saved from the check plots of 1922–1923 are noteworthy

Muscaria botryoides, Narcissus poeticus, Galanthus nivialis, and *Crocus vernus,* except in one case on the last-named species. Ritzema Bos (1903 a:181) observed severe losses in iris, hyacinth, and gladiolus in the field, which he attributes to this fungus.

Westerdijk (1911:26), who grew this fungus in pure culture, records failure to obtain infection of the roots of beets, carrots, and potato tubers by inoculation in moist chambers in the laboratory, although it readily attacked tulip and hyacinth bulbs under the same conditions. She obtained infection with it in one case on onion bulbs.

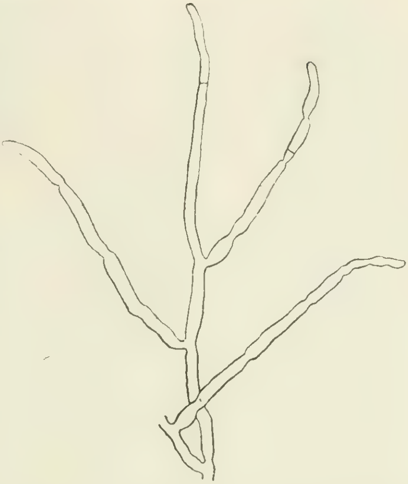


Figure 2. BRANCHING TIP OF RAPIDLY GROWING MYCELIUM IN POTATO AGAR, PETRI-DISH CULTURE

The mycelium is filled with densely granular protoplasm

Life history

All the evidence from the work of previous investigators and from the authors' researches indicates that this pathogene depends upon its sclerotia to tide it over from one season to the next. Mycelium which is readily produced from the sclerotia, spreads through the soil and attacks the suscept. The pathogene appears to be a low-temperature parasite, its attack on the bulbs occurring late in the fall or during the mild weather of winter and early spring. Diseased bulbs always show a well-developed root system which is rarely affected. The attack apparently occurs at the nose, or tip, of the bulb as the shoot begins to protrude from the fleshy scales. Because of the restricted range of suscepts, the disease may be expected to become serious only in soils repeatedly planted to tulips. Since affected bulbs are usually more or less completely destroyed before digging time, and since the sclerotia are largely produced in the soil around diseased bulbs, the chances of its rapid dissemination are slight.

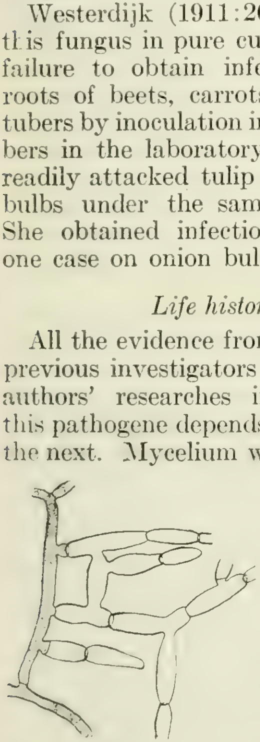


Figure 3. BRANCHING AND ANASTOMOSING MYCELIUM IN OLD CULTURE ON POTATO AGAR

Barrel-shaped cells entirely empty; protoplasm of main branch containing granular vacuolate protoplasm

CONTROL

In order to exclude the pathogene from uncontaminated areas, it is important to know in what manner it is transported from one locality to another. Klebahn (1907:28–29), who gave considerable attention to this matter, concludes that the fungus probably is not often carried on tulip bulbs since infected bulbs are so severely rotted that they would seldom appear in the market. Moreover, he observed that affected bulbs rarely produce bulbils. Both Klebahn and Ritzema Bos suggest that the fungus may be transported in diseased hyacinths or on bulbs of other plants which suffer slightly with the disease. Both Müller-Thurgau (1908:750) and Lendner (1911) cite cases of the occurrence of the disease in Switzerland, in which the pathogene was undoubtedly brought in with diseased bulbs imported from Holland. Lendner observed furthermore that the newly formed bulbils also were attacked. The authors' conclusion on this point, based upon the outbreak of the disease at Yonkers, is that there, at least, all the evidence points to the introduction of the pathogene on the tulip bulbs which were imported from Holland. No other kind of bulbs had been recently planted in these beds. It is indeed probable that the pathogene is only occasionally transported with bulbs in the trade, and this may account for its apparently restricted occurrence. Were it a pathogene commonly transported on tulip bulbs, it would long ago have appeared in America with sufficient frequency and severity to have attracted attention.

The pathogene is a soil-contaminating fungus maintained from year to year by its sclerotia. It appears to spread but slowly year after year from a given center of contamination in the bed, and then only when tulips are planted continuously in the same soil. That it may be disseminated over a plot of ground through spading and cultivation is evident.

The ease with which the sclerotia become separated from the diseased bulbs, their formation in the soil about the bulbs, and the rotting-away of the bulbs before the beds can be reworked and the diseased plants removed, makes sanitary measures such as the removal of diseased bulbs and surrounding soil to appear of little practical value. This seems, however, to be the method generally in vogue among Dutch bulb-growers, who employ for this purpose a special tool (figure 4) known as the Tulpenstecker (tulip sticker).

Rotation where practical is recommended by Klebahn (1907:31), who holds, however, that at least three years must elapse before tulips may safely be returned to soil that has been infested. Moreover, other susceptible bulbs may not be grown therein during the interval (page 26 of same reference).

Ritzema Bos (1903 a:198–202 [p. 350-351 Ztschr.]) tested a number of soil disinfectants for the control of the disease, among these being lime, sulfur, and carbolineum. Of these, only the last proved to be fairly efficient, although a considerable reduction in diseased bulbs was obtained by rolling the bulbs in sulfur after wetting them with a 10-per-cent glycerine solution, together with a liberal use of sulfur placed around the bulbs when planting.

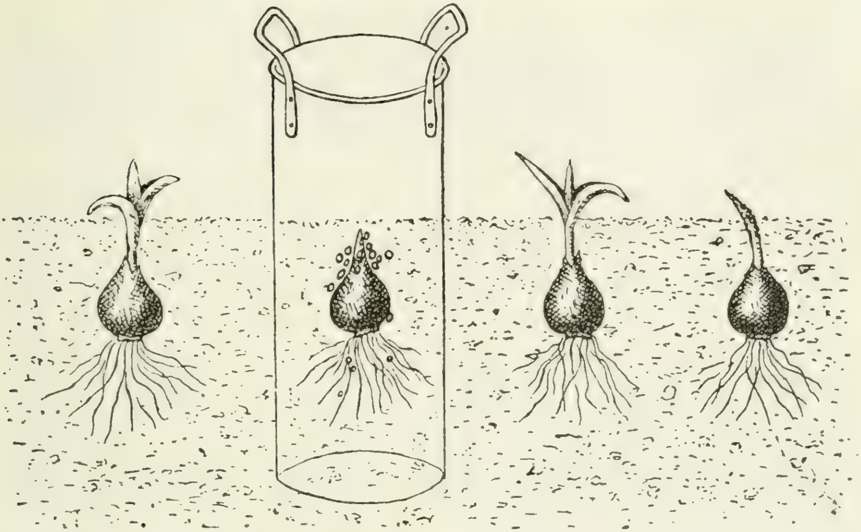


FIGURE 4. "TULPENSTECKER" USED BY THE DUTCH BULB-GROWERS FOR REMOVING DISEASED TULIPS WITH ADJACENT CONTAMINATED SOIL FROM THE BEDS

(From Klebahn, 1907: 30)

The carbolineum was applied at the rate of from 40 to 50 liters to an "are" (= 120 square yards), being first mixed with sand which was then applied and worked into the upper layer of soil. When bulbs were planted immediately after treatment of the soil, rather severe injury resulted especially where the larger amount of carbolineum was applied. Where the treated soil was allowed to lie idle until the next season, no injury resulted, and excellent, though not perfect, control was obtained. Ritzema Bos suggested that treatment of contaminated soil early in the summer, allowing it to lie idle until autumn, and then planting to healthy bulbs, would largely obviate injury from the carbolineum. Klebahn (1906: 562) says that in spite of the work of Ritzema Bos and that of a special commission maintained by the bulb growers of Noordwijk, Holland, no wholly satisfactory method of control had at that time been developed. This is, in his opinion, due largely to their confusing this disease with the Botrytis blight.

Klebahn (1907: 32) recommends a combination of the carbolineum disinfection with the removal of diseased bulbs. The few diseased bulbs that appear after soil disinfection can be easily removed early in the spring before the pathogene has spread to any distance in the soil.

The proximity of the contaminated beds at Yonkers to the Boyce Thompson Institute seemed to offer an excellent opportunity to attempt some experimental work on the control of the disease by means of soil disinfection. The junior author undertook the work of carrying out these experiments in the fall of 1922. The bed in which the experiments were conducted was 66 feet long and 5 feet wide (figure 5). It was bounded on one side by a cement walk and on the other by upright boards about 6 inches wide. This area was divided into six plots. The first five plots were each 10 feet long, and the sixth was 11 feet long. An isolation strip about 1 foot wide was left between adjoining plots. These isolation strips were set off from the adjacent plots by upright board partitions sunk some inches into the soil. Each plot thus contained 50 square feet of planted space, but, since every other plot (nos. 2, 4, and 6) and its adjoining isolation strips were treated with formalin, the treated areas each contained approximately 60 square feet.

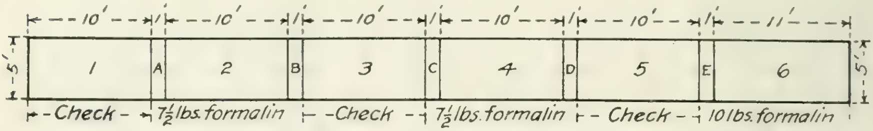


FIGURE 5. PLAN OF THE PLOTS IN THE 1922–1923 CONTROL EXPERIMENTS

Although the disease had appeared only in certain parts of this bed, in preparing the bed in the spring of 1921 an attempt was made to thoroughly distribute the pathogene before dividing the bed into plots The diseased bulbs had not been removed in any numbers but had been allowed to rot down, and presumably an abundance of sclerotia were formed.

Plots 1, 3, and 5 (figure 5) were left untreated as checks. To plot 2 with its adjoining isolation strips A and B, and to plot 4 with its adjoining isolation strips C and D, was applied 7½ pounds of 40-per-cent formalin each, diluted with water at the rate of 1 to 50. Plot 6 with its adjoining isolation strip E received 10 pounds of formalin diluted with water at the rate of 1 to 50. The solution was applied as evenly as possible in each case, and the bed was then covered with tarred paper. The soil had been wet by a rain the day before treating.

The formalin was applied on October 11, 1922. The tarred paper was removed on October 23. The bulbs purchased from importers were first peeled, that is, the dry brown scales were removed. All cankers or diseased spots in the fleshy outer scales were carefully cut out, and the bulbs were then disinfected by soaking for one hour in a 1-to-120 solution of formalin, after which they were rinsed through three changes of tap water and spread out to dry.

The bulbs were planted in four rows lengthwise of the plots, none being set in the isolation strips. There were approximately fifty bulbs in each plot. The check plots 1, 3, and 5 were planted first, the treated plots meanwhile being covered with tarred paper to prevent accidental transfer of contaminated soil to them. The check plots were then covered and the treated plots were planted, after which all plots were left exposed.

On April 17, 1923, the junior author made the following report: "Plot 6 shows practically a complete stand — only two tulips are missing in the entire bed. Plots 2 and 4 show about one-half a stand of tulips. The control plots 1, 3, and 5 do not show more than three or four tulips in each." (Plate VI).

A short time later the senior author inspected the plots and found the visual results even more striking than the report.

It is evident that to effectively disinfect soil contaminated with this pathogene, at least 1 pound of formalin (40-per-cent) must be applied to every 6 square feet of soil. This makes the treatment rather expensive, but it is effective and for small areas is not prohibitive. If the "bad spots" are marked as soon as discovered and are promptly treated, it should rarely be necessary to disinfect large areas. Since steam sterilization should be even more effective, a series of such treatments was made on the same area in the fall of 1923. The experiment (figure 6) was laid out as follows: The plots were the same as those used in the previous season's (1922) experiments and of the same size and arrangement (figure 5). Before treatment, contaminated soil from the check plots of the previous year was thoroughly mixed with the soil in the other plots. Plots 1 and 6 were left untreated as checks. Plots 2, 3, and 4 were steam-sterilized by the steam-pan method — plot 2 for one-half hour, plot 3 for one hour, and plot 4 for two hours. Plot 5, along with isolation strip D, was treated with formaldehyde solution, 15 pounds diluted in water at the rate of 1 to 50. Four plots were planted with healthy tulip bulbs (variety Moonlight) on November 17, 1923. Plot 5 was not planted until December 3.

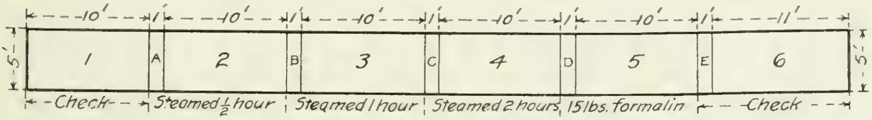


FIGURE 6. PLAN OF THE PLOTS IN THE 1923–1924 CONTROL EXPERIMENTS

On June 10, 1924, the junior author reported that the plants in check plot 1 were badly injured by the disease. Of the 35 bulbs planted in this plot only 13 plants came up, of which 11 showed evidences of disease; 8 of these plants bloomed.

Fifty-two bulbs were planted in each of the steam-sterilized plots. Plot 2 gave a stand of 50 plants, of which only 4 showed disease; 27 bloomed. Plot 3 gave a stand of 50 plants, of which 3 were diseased; 25 bloomed. Plot 4 showed a stand of 47 plants, of which 6 showed disease; 22 bloomed.

The small proportion of blooming plants in these plots in 1923 is to be attributed largely to weak bulbs and not to the disease.

It will be noted that in all three of the steam-sterilized plots (2, 3, and 4) a nearly perfect stand was obtained (Plate VII) and the proportion of diseased plants was approximately the same, 3 to 6 plants to the bed. This indicates that half an hour's steaming is as effective as the longer treatments.

Plot 5, treated with formaldehyde solution, gave a stand of 49 plants, of which 8 showed disease and all blossomed (Plate VIII).

Plot 6, the other check plot, gave a stand of 41 plants out of 52 planted, but of these 21 showed disease; 15 bloomed (Plate VIII). This plot had never shown a high percentage of disease, due apparently to a tile drain across it which tends to keep the soil drier than in the other plots.

These results indicate clearly that the bulb-rot pathogene may be largely eradicated and a good stand obtained by soil disinfection with formalin at the rate of 1 to 1½ pounds to 5 or 6 square feet of soil. The formalin should be diluted in enough water to thoroughly wet down through from 6 to 8 inches of loosely spaded soil. Pan steaming for from one-half to one hour also is effective.

In the control, or check, plots in the spring of 1923, a few plants, as already pointed out, came through with no trace of disease. It appeared possible that these individuals were resistant or immune to the pathogene. In order to test this possibility, these plants were dug after maturity and saved for planting in the contaminated soil in the experimental plots that autumn. The bulbs were planted in two extra rows on either side of plot 6, one of the checks for the experiment of 1923–24. Practically every one of these bulbs grew and produced flowers in the spring of 1924, and again showed no trace of the disease. This indicates rather strongly that they are resistant or immune. Thus a strong probability exists that resistant or immune strains of tulip varieties may be obtained by the selection and propagation of disease-free individuals from populations growing in badly contaminated soil.

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CORNELL UNIVERSITY

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KARL HERMAN FERNOW

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INTERSPECIFIC TRANSMISSION OF MOSAIC DISEASES OF PLANTS[1]

Karl Hermann Fernow

The subject of mosaic diseases is one of great and increasing importance in plant pathology. A large number of economic plants and many wild species are known to be affected. The question of the intercommunicability of mosaic diseases among different suscepts is still largely an unanswered one. Many reports in literature on attempts to transmit them are merely incidental to other investigations, and leave the reader in doubt as to the methods employed and as to the extent of the experimental work on which conclusions are based. In many cases reports of different investigators are in conflict.

The purpose of the studies here reported is to determine: (1) whether the mosaics of different plants are due to distinct causes, here called *viri*, or whether all are due to a single virus; (2) if the causes are several, what plants are affected by each; and (3) what are the symptoms produced by the different viri on various suscepts. Obviously it is impossible to fully answer the above questions in an inquiry as brief as this.

The experiments described were carried on in the greenhouses of the Department of Plant Pathology at Cornell University, and on the experimental farm, during the years 1922, 1923, and 1924.

ACCOUNT OF EXPERIMENTAL WORK

MATERIALS

Source of inoculum

For the sake of convenience, the various sources of inoculum are designated by the letters A to H. As is shown later, these different sources represent also different viri, at least in part. The original sources of the inoculum designated by these letters were respectively as follows:

A. Juice of mosaic tobacco plants kept in a bottle in the greenhouse for several years but still found to be infective.

B. Mosaic potato plants grown from tubers collected in the field in various parts of New York. Also, plants grown from tubers sent to the writer by Dr. Donald Folsom, of Maine, which represent the various types of mosaic described by Schultz and Folsom (1923). Although these types appear very different on the potato, the writer was not able to distinguish any difference in the symptoms on other species when inoculated from potato.

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[1] Also presented to the Faculty of the Graduate School of Cornell University, June, 1925, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

C. Mosaic *Datura Stramonium* L. plants sent to the writer by Dr. A. F. Blakeslee from Long Island.

D. A *Nicotiana glutinosa* L. plant which developed mosaic after inoculation from a *Datura Stramonium* plant affected with mosaic C. While this would seem to indicate that mosaics C and D are identical, the data on this point are rather inconclusive and it seems better to regard these mosaics as distinct until proof can be furnished that they are identical.

E. *Phytolacca decandra* L. plants grown from roots of mosaic plants dug near Tully, New York. In all cases the inoculum was taken from the original plants.

F. Mosaic *Rumex obtusifolius* L. plants found near Ithaca and transplanted to the greenhouse.

G. Mosaic *Phaseolus vulgaris* L. plants grown in the greenhouse from infected seed. Inoculum for the field experiments was taken from plants of unknown variety growing in experimental plots.

H. Mosaic *Echinocystis lobata* (Michx.) Torr. & Gray plants growing outdoors in Ithaca.

Plants inoculated

The species selected for inoculation were chosen for various reasons. Some were used because of their relationship to affected plants available, or because of mention in literature. It was naturally impossible to work with a large variety of species. Certain species were discarded because of difficulty of culture or the failure to obtain infection.

Except in the case of potatoes, all the plants used were grown from seed. Special efforts were made to keep the plants growing vigorously by frequent repotting. It was found that some species grew so slowly in the short days of winter that they were not satisfactory for inoculation.

The only inoculations made out of doors were made in the summer of 1923. The plants were started in the greenhouse and transplanted to the field, being spaced three feet each way and one row of sixty-six plants of each species being grown. Some species were repeated.

It was thought that more reliance could be placed on results obtained repeatedly with a relatively small number of plants, than on results obtained with a larger number of plants all inoculated on one date. It seemed desirable also to make the inoculations with different mosaics on the same species on the same date, so that the results would be comparable. As space did not permit the growing of very large numbers of one species at one time, it was necessary to reduce the number of plants in .each experiment. In some cases this was doubtless carried to too great an extreme, as when a single plant was inoculated from each source of inoculum. From three to five plants were inoculated from each source of inoculum at one time in most of the greenhouse experiments, and six or seven plants were used in each experiment in the field.

Generally, as many checks were left as there were plants inoculated with each source of inoculum. The checks were usually treated in the same manner as the inoculated plants, except that a leaf of the check plant itself was substituted for the inoculum. With potatoes, the procedure was different. In planting, a tuber was cut in several (from 4 to 10) pieces and planted in as many pots, each being given the same number but a different letter. All the plants except one were used in inoculation experiments. This one was saved as a check for freedom from disease of the whole unit. For the most part, these were the only checks used for potatoes. The second vegetative generation was always grown when tubers could be obtained. In most of the inoculations made on potatoes, the plants included in any one experiment were taken from different tuber units.

METHODS OF INOCULATION

Rubbing

The method of inoculation used in most of the earlier experiments was as follows: A mosaic leaf was crushed in the hands into a little ball. A leaf of the plant to be inoculated was held in the left hand and vigorously rubbed with the crushed tissue held in the right hand. Sometimes the inoculation was made with one hand only, rubbing the leaf and the inoculum together between the thumb and the forefinger. The rubbing was vigorous enough to break the epidermis of the leaf and was repeated on several leaves of each plant inoculated. Under these conditions the method was found very effective. The hands were freed from infective material by washing with soap, as recommended by Allard (1917).

A variation of this method consisted in the wearing of rubber gloves. The gloves were disinfected by soaking in 10-per-cent formalin for about five minutes. This method was soon abandoned.

The method first described was likewise abandoned early in the course of the experiments, in favor of the following: Two pairs of forceps were used, one with curved points and one with broad flattened points. The forceps were flamed and then used to fold the leaf several times. The inoculum was then held in the curved forceps and rubbed on the leaf to be inoculated, which was supported from behind by the flat-pointed forceps.

In the later experiments the inoculum was sometimes ground in a mortar, which had been disinfected by standing in sulfuric acid and rinsed in water. In this case a knife blade was generally substituted for the flat-pointed forceps, as a support for the inoculated leaf.

Split-stem method

In a few experiments, a slit was made in the stem of the plant to be inoculated, and crushed mosaic leaf tissue was inserted. Flamed instruments were used. This method, however, was found less effective than rubbing.

Grafting

When other methods fail, positive results may sometimes be obtained by grafting. This method was used especially with potatoes. There are, however, several drawbacks. The method is laborious; the scion often fails to live, and, even if it does live, it usually makes but little growth; inoculum carried on the hands or on the knife may be accidentally introduced; furthermore, only those species can be grafted which are closely related. The procedure found most satisfactory was that known as bottle grafting (Blakeslee and Farnham, 1923). This is essentially inarching, the lower end of the scion being placed in a shell vial containing water to prevent drying out.

Comparison of different methods

It was soon found that the methods involving touching the plants with the hands were unsatisfactory because inoculum was thus carried from one experiment to the next. This is well shown in a series of experiments, the results of which are given in table 1. Mosaic material was handled or was placed on the instruments prior to the treatment of each plant. The hands or instruments were then disinfected and the healthy plant was inoculated with its own leaves. The results would seem to indicate that, of the methods of disinfection tried, the flaming of instruments was the only dependable one. The results would seem to indicate also that the method of inoculation involving the placing of inoculum in a slit

TABLE 1. INOCULATION OF TOBACCO PLANTS BY DIFFERENT METHODS

Inoculum	Methods		Number of plants inoculated	Results
	Inoculation	Disinfection		
Mosaic	Hands	None	2	All mosaic
Own leaves	Hands	Soap	4	All mosaic
Mosaic	Rubber gloves	None	4	All mosaic
Own leaves	Rubber gloves	10-per-cent formalin, 2 minutes	2	All mosaic
Own leaves	Rubber gloves	10-per-cent formalin, 5 minutes	3	2 mosaic
Own leaves	Rubber gloves	10-per-cent formalin, 10 minutes	1	All mosaic
Mosaic	Stem slit		4	All mosaic
Own leaves	Stem slit	Flame	4	All healthy

in the stem was effective. Subsequent experiments showed this method to be less reliable, as well as more cumbersome, than the rubbing method.

As a rule, the instruments were disinfected after inoculation of each plant. By this procedure, if one of the inoculated plants was already affected with mosaic without showing the symptoms, the disease would not be carried from this plant to the others. In some cases in which larger numbers of plants were used, the instruments were flamed only after inoculation of two or three plants. In the earlier grafting experiments, mosaic might have been carried from plant to plant by the razor used in grafting; but later this danger was obviated by using safety-razor blades and discarding the blades after they had been used on one plant.

RECORDS

For the purpose of keeping records, all the plants of one species inoculated with the same source of inoculum at one time were assigned a number. Each plant was given a letter to distinguish it from the other plants of the same experiment. The plants were examined almost daily, and a record was kept of the first symptoms observed on each plant.

DIAGNOSIS

Ordinarily, no method of diagnosis was used other than the visual examination of the plants for symptoms. This method, of course, leaves something to be desired with reference to negative evidence. It is quite possible for the symptoms of a mosaic on a given suscept to be so obscure, at least under some conditions, as to escape detection; in such a case, reinoculation of the original suscept from the plants in question would show that they were carriers. It was unfortunately not found practicable to carry out this method, mainly because the limited space available made it impossible to keep on hand a constant supply of the necessary suscepts in vigorous condition.

It is possible also to err in the other direction, and diagnose as affected by mosaic a plant which has been injured by fumigation, insects, or soil conditions. There may have been isolated instances wherein this mistake was made, but usually mosaic was not recorded unless definite and unmistakable symptoms were present. Extremely doubtful cases were omitted altogether from the tables.

Recognition and description of different mosaics

As already mentioned, the different sources of inoculum produce different effects on the same suscepts. It is convenient at this point to describe the symptoms produced by the different viri on the various species used. In keeping the records of the experiments, it was found desirable to record not only the presence or the absence of mosaic, but also, in positive cases, to record which mosaic was present. It will readily be understood that the chances of error in this matter are greater than in the simple statement as to whether or not a plant is affected with mosaic. Two mosaics on the same species may be very distinct in their characteristic expression and yet individual cases will occur in which it is difficult or impossible to say with which mosaic one is dealing. In the brief discussions of the various mosaics which follow, it must be borne in mind that the symptoms vary greatly with the conditions, not only in intensity but even in kind. To describe all the possible symptoms that may be produced by each mosaic would require very lengthy descriptions preceded by years of experience. Even the best of such would prove inadequate to the recognition of these mosaics by persons not already acquainted with them. The descriptions are therefore largely comparative, and are presented for the purpose of distinguishing between the various mosaics when two or more are at hand on the same species. It will be found useful to refer to the accompanying plates for more ready comparison.

Datura meteloides Dunal and *D. Stramonium* appear to be subject to both mosaic B and mosaic C. The symptoms of mosaic B (Plate I, 2) are usually rather large irregular areas of yellowish green appearing on the dark green background of the leaf, with little or no rugosity of the leaf. Mosaic C (Plate I, 3), on the other hand, produces a very pronounced rugosity, often causing the leaf to appear blistered, and the leaves are not infrequently narrowed and twisted. The flowers are sometimes distorted, and are generally marked with blue or purple streaks on the purple variety. It is not certain whether the streaking may not occur also in connection with mosaic B.

Lycopersicon esculentum Mill. is affected by both mosaic A and mosaic B. The symptoms of mosaic A (Plate I, 6) are well known, this probably being the mosaic of tomatoes generally referred to in literature. The leaves show rather sharply contrasted areas of dark green and light yellowish green. The mottling is usually most readily detected on the very young leaves. It is often somewhat difficult to detect mosaic on tomato, because of the narrowness of the leaves and the fact that the young leaves are often normally lighter-colored and somewhat distorted. The distortion due to mosaic A is usually greatest in plants that have become infected when very small. In such plants, the blade of the leaf may be almost entirely suppressed, and the filiform leaves much twisted and curled. In artificially inoculated plants, the distortion is generally not so striking.

The symptoms of mosaic B (Plate I, 5) on this plant are much less striking. As the leaves become fully expanded they show a rather ill-defined mottling. The symptoms may be easily mistaken for the effects of soil or water conditions or of unfavorable temperature. As far as observed, the very young leaves show no noticeable symptoms and no appreciable distortion. The contrast between these two mosaics of tomato is much greater than would appear from an examination of Plate I (4, 5, and 6).

The mosaic on *Martynia louisiana* Mill. shows as a distinct mottling affecting rather large, well-defined patches.

Nicandra Physalodes (L.) Pers. is affected by two mosaics. Mosaic B (Plate II, 8 and 11) produces a distinct mottling consisting of small light areas in the darker leaf. Generally the leaves are not much distorted but often they are somewhat smaller than those of healthy plants and they are sometimes slightly cupped, the edges turning downward. They may be slightly rugose. The flowers are not affected except that they also may be smaller than normal. Mosaic H on *N. Physalodes* (Plate II, 10) produces almost no mottling, but instead one finds a pronounced and characteristic distortion. The leaves develop very irregular lacerated margins and become more pointed. The veins are more prominent and straighter, and branch in a peculiar manner. The flowers are often much affected (Plate III). They are almost always smaller than normal, the sepals often show the peculiarities of venation described for the leaves, and the corolla is often divided. The divisions of the corolla do not always correspond to the normal petal lobes, and may divide it into two or three parts of unequal size.

Three mosaics occur on *Nicotiana glutinosa*. Mosaic B (Plate IV, 17 and 19) causes a fine mottling consisting of light green islands with darker green showing along the smaller veins. Usually the mottling is not very striking under greenhouse conditions, but it may be so in the field where the plants grow rapidly. Mosaic D (Plate IV, 16 and 20) shows as a very faint spotting. There are generally only four or five of these small spots on each leaf. They are usually not clearly outlined and may be evident only by transmitted light. Mosaic H (Plate IV, 14 and 15) is extremely pronounced and distinct, in contrast to the other mosaics occurring on this species. The affected leaves show large contrasted areas of yellowish green, and sometimes dark green blisters, as in the case of the small leaf in Plate IV, 14. The leaves are generally somewhat dwarfed and distorted, and sometimes they are much reduced. The appearance of this mosaic is similar to that of the familiar mosaic on tobacco.

Mosaic A on *Nicotiana rustica* L. (Plate VI) has a striking and unusual effect. The leaves generally turn yellow and then brown near each point of inoculation. The leaves near the growing point then turn yellow, growth ceases, and the terminal bud and the younger leaves die and shrivel up, leaving only the stump and some of the older leaves green. On splitting the stem, it is seen that necrotic spots occur in the interior.

Mosaic A on *Nicotiana Tabacum* L. (the well-known tobacco mosaic) is characterized by a distinct mottling consisting generally of rather large yellowish green areas intermingled with large patches of dark green. Sometimes the lighter areas predominate, but usually the darker part is more extensive. Sometimes the darker areas appear as blisters. Not infrequently, if the plant is affected early, the leaves may be considerably distorted. The flowers also have been observed to be distorted and abnormally pigmented, as described by Allard (1914).

Mosaic G on *Phaseolus vulgaris* (Plate V, 21) is generally manifested by a mottling consisting of rather large, well-defined patches of light and dark green. The younger leaves are often reduced in size, and cupped.

Mosaic A on *Physalis heterophylla* Nees is similar in appearance to the ordinary tobacco mosaic.

Mosaic A on *Physalis subglabrata* Mackenzie & Bush (Plate II, 12) is also similar in appearance to tobacco mosaic.

Mosaic E on *Phytolacca decandra* is similar in appearance to tobacco mosaic, but the leaves are seldom blistered. The mottled areas are generally large.

Mosaic F on *Rumex obtusifolius* shows as a rather fine, regular mottling, not very striking.

On *Solanum aculeatissimum* Jacq. (Plate V, 22) both mosaic A and mosaic B occur. Neither ordinarily produces striking symptoms, and the writer has thus far been unable to distinguish between them with any degree of certainty. It would appear that the contrasted areas were generally larger for mosaic A than for mosaic B.

Solanum atropurpureum Schrank and *S. carolinense* appear to be subject to both mosaic A and mosaic B. As in the case of *S. aculeatissimum*, the two mosaics are not readily distinguished, but mosaic A seems to produce larger contrasted areas than does mosaic B.

Solanum nigrum L. has reacted positively to mosaic A only, in the few experiments performed. The mosaic produced is rather striking, and resembles mosaic A on tobacco.

Solanum tuberosum L. has been found to be subject to both mosaic A and mosaic B. Inoculations with mosaic A by grafting produce very remarkable symptoms. The young shoots of the potato are affected with what was at first thought to be an attack of early blight. Small yellowish spots show on the leaves. These spots soon die and turn brown. The petioles and the young stems become affected with a severe necrotic streaking and the affected shoots generally die soon after. Sometimes no symptoms appear until the second vegetative generation. The second-generation plants are usually much dwarfed (Plate VII, 25) and the leaves may show from one to five yellowish spots which soon become brown. As these spots often appear before the leaf has fully expanded, and as the affected areas are unable to grow, the leaf develops a puckered appearance. The petioles and stems are often affected with a more severe necrotic streaking than in the first-generation plants, and the older leaves are dropped, leaving only a small crown. These symptoms closely resemble those described for potato streak by Schultz and Folsom (1923).

Mosaic B was obtained whenever potatoes were used as a source of inoculum on a suitable suscept. This was the case whether the potatoes used showed symptoms of mild, leaf-rolling, or rugose mosaic (Schultz and Folsom, 1923), mosaic A, or some other virus disease. Later the same symptoms were obtained when apparently healthy potatoes were used as sources of inoculum. One is therefore led to the conclusion that the symptoms of the various mosaics mentioned are due not to mosaic B but to other viri, and that the virus of mosaic B produces no visible symptoms on potato.[2]

Time required for the production of symptoms

The length of time after inoculation and before symptoms appeared varied greatly with the suscept, the inoculum, and the environmental conditions. In general, the symptoms appeared most quickly on plants that were growing rapidly. The shortest time recorded was four days; this was for *Nicotiana rustica* inoculated with mosaic A. Other plants showed the first detectable symptoms seven weeks after inoculation, and with potatoes the symptoms were sometimes detected only in the second generation, seven months or more after inoculation. It may be said that the longer periods usually indicate conditions unfavorable for the detection of mosaic, such as slow-growing pot-bound plants or red-spider or thrips injury. Under favorable conditions of temperature and other factors, plants will usually show symptoms within three weeks. In any experiment, the individual plants are likely to show mosaic at about the same time. Plants were therefore generally kept for only a week or so after the first symptoms began to appear.

One would naturally expect to find some difference in the length of time between inoculation and the appearance of symptoms, according to the suscept and the inoculum. It is evident that so many other factors were involved, besides these two, that conclusions drawn from the experiments here reported can be regarded only as approximations. However, the general impression gained from experience is that in most cases the period varies more with the suscept than with the inoculum used, and that there is a considerable personal equation involved; that is to say, one person will recognize the first symptoms more readily than will another, and the same person will recognize early symptoms more readily after some experience with a given mosaic and suscept than at an earlier date in his experience.

When inoculated with mosaic A, *Nicotiana rustica* appears to show symptoms soon, tobacco and *Solanum aculeatissimum* somewhat later, tomato still later, and potato only after a considerable period. When inoculated with mosaic B, *Datura Stramonium*, *Solanum aculeatissimum*, *Nicandra Physalodes*, and *Nicotiana glutinosa*, show symptoms readily, while tomato and potato are slower. *Datura Stramonium* seems to show mosaic B more quickly than mosaic C, and *Nicotiana glutinosa* appears to show mosaic B only slightly more quickly than mosaic D. Mosaic H apparently develops very slowly on both *Nicandra Physalodes* and *Nicotiana glutinosa*. However, another investigator with a different point of approach might recognize earlier symptoms on the suscepts here mentioned as slow, or might overlook early symptoms on suscepts here mentioned as showing symptoms early.

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[2] This phenomenon is discussed at greater length on page 31.

SOURCES OF ERROR

Any series of experiments such as is here reported is subject to certain sources of error. The errors may be classified as errors in diagnosis, errors due to accidental contamination, and errors due to the application of methods which are ineffective for the particular disease and species of plants under consideration.

Errors of diagnosis

Errors of diagnosis may be in either direction. There is abundant evidence in literature, as well as in the writer's experience, that plants may, under some conditions, harbor mosaic for long periods without showing any visible symptoms. Such cases are reported for eggplant by Melhus (1922), and for *Physalis Alkekengi* L. by Nishimura (1918). If the plants are kept rapidly growing under favorable conditions, there is less likelihood of error from this cause. The feeling is general among plant pathologists that negative results are of little significance as compared with positive results. While this feeling is justified in general, it must not be forgotten that positive results may also be misleading. Positive results may be reported in the case of plants not actually affected, due to the effects of low or high temperatures, insufficient water, infestation with thrips or red spider, or other conditions, or they may be due to natural infection.

In addition to positive diagnosis of plants affected with mosaic, some attempt should always be made to identify the mosaic concerned. It is evident that very few authors have made any attempt to determine whether all the plants of a given species which they succeeded in artificially infecting were attacked by the same virus. This would necessitate the inoculation of parallel series of plants and a careful comparison of the infected plants. Even when this is done, the chances of an error in the identification of the mosaic are greater than the chances of an error in determining whether the plant in question is affected with mosaic.

Errors due to accidental contamination

There are a number of ways in which contamination may occur. These may be briefly mentioned:

1. The plant used as the source of inoculum may be affected with two or more mosaics.
2. The inoculum may become contaminated during the process of preparing it for use.
3. The instruments (or hands) used in making the inoculation may be contaminated.
4. The inoculated plant may become infected from some source other than the experimental inoculum. Ways in which this may occur are: by insects; by handling by the experimenter or by visitors; by contact of the injured parts of the plants, as the rubbing together of inoculated leaves or the breaking and rubbing of roots in moving the pots; and by the use of plants from previous experiments where symptoms are masked.

Errors due to the use of ineffective methods

It is well within the bounds of possibility that methods which would give good infection with one mosaic or a certain suscept, would fail with a different combination of mosaic and suscept. For example, it is well known that tobacco mosaic can be transmitted by hypodermic injections. Reddick and Stewart (1919) were unable to transmit bean mosaic by this method, although they made many attempts.

RESULTS OF EXPERIMENTS

The results of the inoculations with material from different species are presented in tables 2 to 20. These data are summarized in table 21 with respect to the particular mosaic concerned.

TABLE 2. INOCULATIONS ON DATURA METELOIDES

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A..........	*Nicotiana Tabacum*.................	3	0	....
B..........	*Solanum tuberosum*..................	7	5	Uncertain
C..........	*Datura Stramonium*.................	7	4	Uncertain
D..........	*Nicotiana glutinosa*.................	7	2	Uncertain
E..........	*Phytolacca decandra*................	3	0	....
F..........	*Rumex obtusifolius*..................	5	0	....
G..........	*Phaseolus vulgaris*..................	3	0	....
H..........	*Echinocystis lobata*..................	7	0	....
Checks.....	Own leaves..........................	7	0	....

* For the original source of the mosaic indicated by letters A to H, see pages 3 and 4.

TABLE 3. INOCULATIONS ON DATURA STRAMONIUM

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Lycopersicon esculentum*	6	0	
A	*Nicotiana Tabacum*	11	3	C
A	*Solanum aculeatissimum*	2	0	
B	*Datura Stramonium*	8	8	B
B	*Lycopersicon esculentum*	6	4	B
B	*Nicandra Physalodes*	3	3	B
B	*Nicotiana glutinosa*	8	7	5 B 2 Uncertain
B	*Solanum aculeatissimum*	1	1	B
B	*Solanum atropurpureum*	5	4	Uncertain
B	*Solanum tuberosum*	29	28	20 B 8 Uncertain
C	*Datura Stramonium*	27	20	4 B 10 C 3 B and C 3 Uncertain
C	*Solanum aculeatissimum*	1	1	C
D	*Nicotiana glutinosa*	23	10	9 C 1 Uncertain
E	*Phytolacca decandra*	10	0	
F	*Rumex obtusifolius*	14	0	
G	*Phaseolus vulgaris*	9	0	
H	*Echinocystis lobata*	6	0	
H	*Nicandra Physalodes*	5	0	
H	*Nicotiana glutinosa*	2	0	
Checks		28	0	

* See footnote to table 2, page 13.

TABLE 4. INOCULATIONS ON LYCOPERSICON ESCULENTUM

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Lycopersicon esculentum*	26	24	A
A	*Nicotiana Tabacum*	59	58	A
A	*Solanum nigrum*	2	2	A
A	*Solanum tuberosum*	3	3	A
B	*Lycopersicon esculentum*	6	5	B
B	*Datura Stramonium*	1	0	....
B	*Solanum tuberosum*	55	41	1 A 27 B 13 Uncertain
C	*Datura Stramonium*	9	0	....
D	*Nicotiana glutinosa*	7	0	....
E	*Phytolacca decandra*	3	0	....
F	*Rumex obtusifolius*	8	0	....
G	*Phaseolus vulgaris*	3	0	....
H	*Echinocystis lobata*	7	0	....
Checks	....	39	0	....

* See footnote to table 2, page 13.

TABLE 5. INOCULATIONS ON MARTYNIA LOUISIANA

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Nicotiana Tabacum*	4	4	Uncertain
B	*Solanum tuberosum*	4	0	....
D	*Nicotiana glutinosa*	4	2	Uncertain
E	*Phytolacca decandra*	5	0	....
F	*Rumex obtusifolius*	4	0	....
G	*Phaseolus vulgaris*	4	0	....

* See footnote to table 2, page 13.

TABLE 6. INOCULATIONS ON NICANDRA PHYSALODES

Mosaic*	Suscept, source of inoculum,	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A..........	*Lycopersicon esculentum*............	11	0	....
A..........	*Nicotiana Tabacum*................	18	4	B
A..........	*Solanum aculeatissimum*............	6	2	B
B..........	*Datura Stramonium*................	1	1	B
B..........	*Lycopersicon esculentum*............	6	6	B
B..........	*Nicandra Physalodes*...............	18	17	B
B..........	*Nicotiana glutinosa*................	3	3	B
B..........	*Solanum aculeatissimum*............	5	5	B
B..........	*Solanum tuberosum*................	116	94	B
C..........	*Datura Stramonium*................	15	0	....
D..........	*Nicotiana glutinosa*................	16	0	....
E..........	*Phytolacca decandra*................	7	0	....
F..........	*Rumex obtusifolius*.................	11	0	....
G..........	*Phaseolus vulgaris*.................	7	0	....
H..........	*Echinocystis lobata*.................	17	10	H
H..........	*Nicandra Physalodes*...............	14	3	H
H..........	*Nicotiana glutinosa*................	17	3	H
H..........	*Nicotiana rustica*..................	2	2	H
Checks.....	....	65	1	B

* See footnote to table 2, page 13.

TABLE 7. INOCULATIONS ON NICOTIANA GLUTINOSA

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Lycopersicon esculentum*	4	1	B
A	*Nicotiana Tabacum*	18	0	
A	*Solanum aculeatissimum*	4	1	Uncertain
A	*Solanum tuberosum*	2	0	
B	*Datura Stramonium*	3	1	B
B	*Nicandra Physalodes*	2	2	B
B	*Nicotiana glutinosa*	4	2	B
B	*Solanum aculeatissimum*	1	1	B
B	*Solanum tuberosum*	52	44	B
C	*Datura Stramonium*	26	3	1 D 2 B
D	*Nicotiana glutinosa*	37	25	1 B 17 D 7 Uncertain
E	*Phytolacca decandra*	8	0	
F	*Rumex obtusifolius*	13	0	
G	*Phaseolus vulgaris*	7	0	
H	*Echinocystis lobata*	7	0	
H	*Nicandra Physalodes*	6	4	H
H	*Nicotiana glutinosa*	4	0	
Checks		21	0	

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* See footnote to table 2, page 13.

TABLE 8. INOCULATIONS ON NICOTIANA RUSTICA

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Lycopersicon esculentum*	4	3	A
A	*Nicotiana Tabacum*	27	25	A
A	*Solanum aculeatissimum*	1	0	....
B	*Datura Stramonium*	2	0	....
B	*Nicandra Physalodes*	3	0	....
B	*Nicotiana glutinosa*	3	1	A
B	*Solanum aculeatissimum*	1	0	....
B	*Solanum tuberosum*	14	0	....
C	*Datura Stramonium*	12	0	....
D	*Nicotiana glutinosa*	11	0	....
E	*Phytolacca decandra*	8	0	....
F	*Rumex obtusifolius*	11	1	A
G	*Phaseolus vulgaris*	6	0	....
H	*Echinocystis lobata*	6	0	....
H	*Nicandra Physalodes*	7	0	....
H	*Nicotiana glutinosa*	2	0	....
Checks	....	23	1	A

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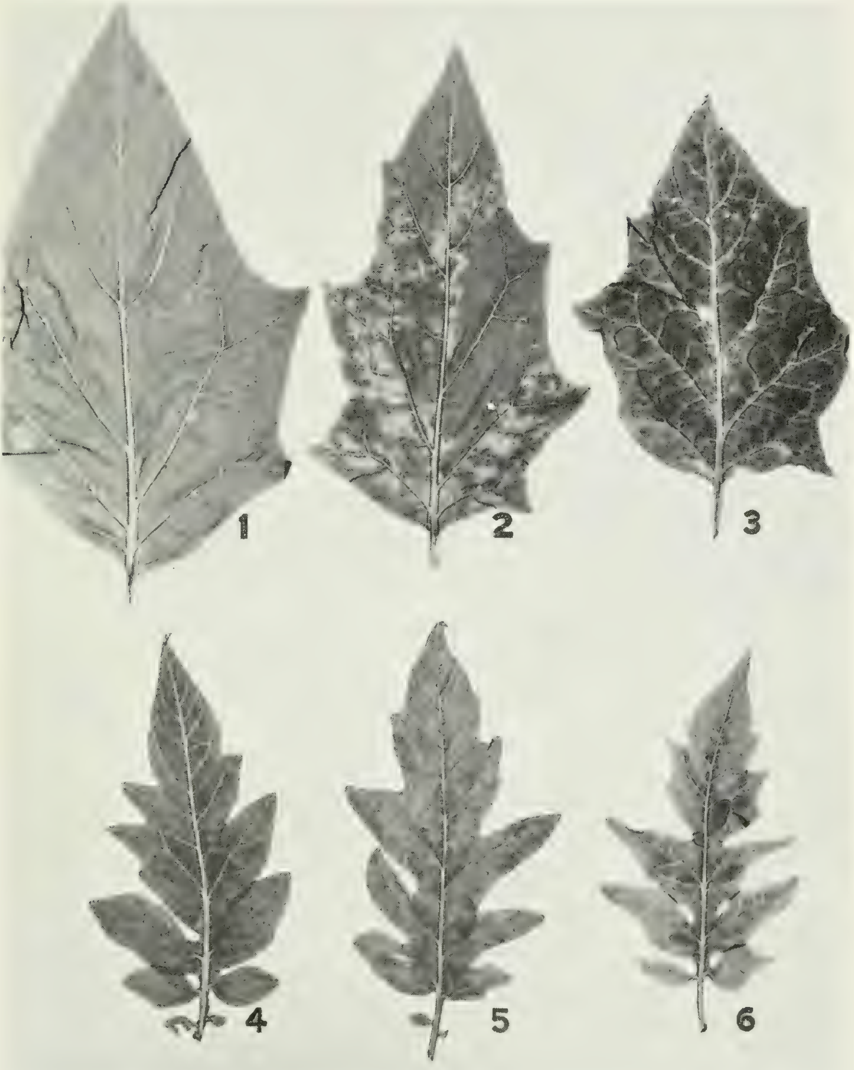
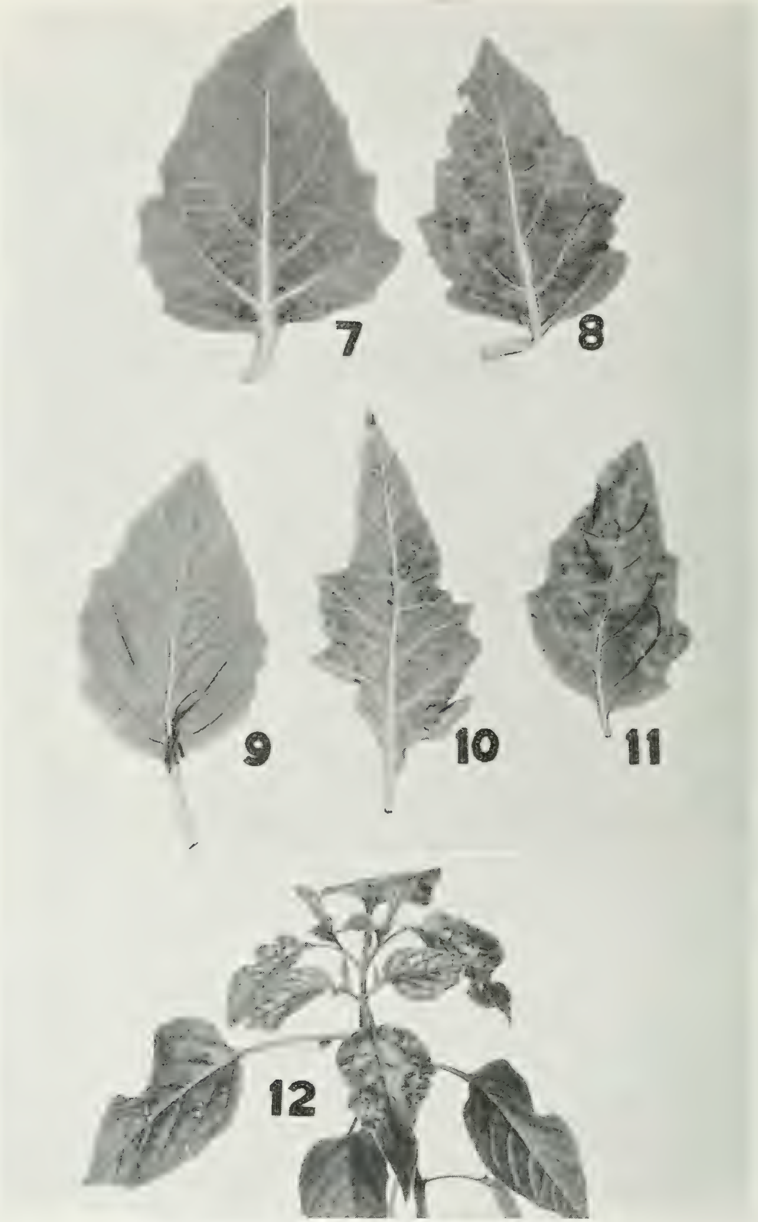
* See footnote to table 2, page 13.



DATURA STRAMONIUM AND LYCOPERSICON ESCULENTUM

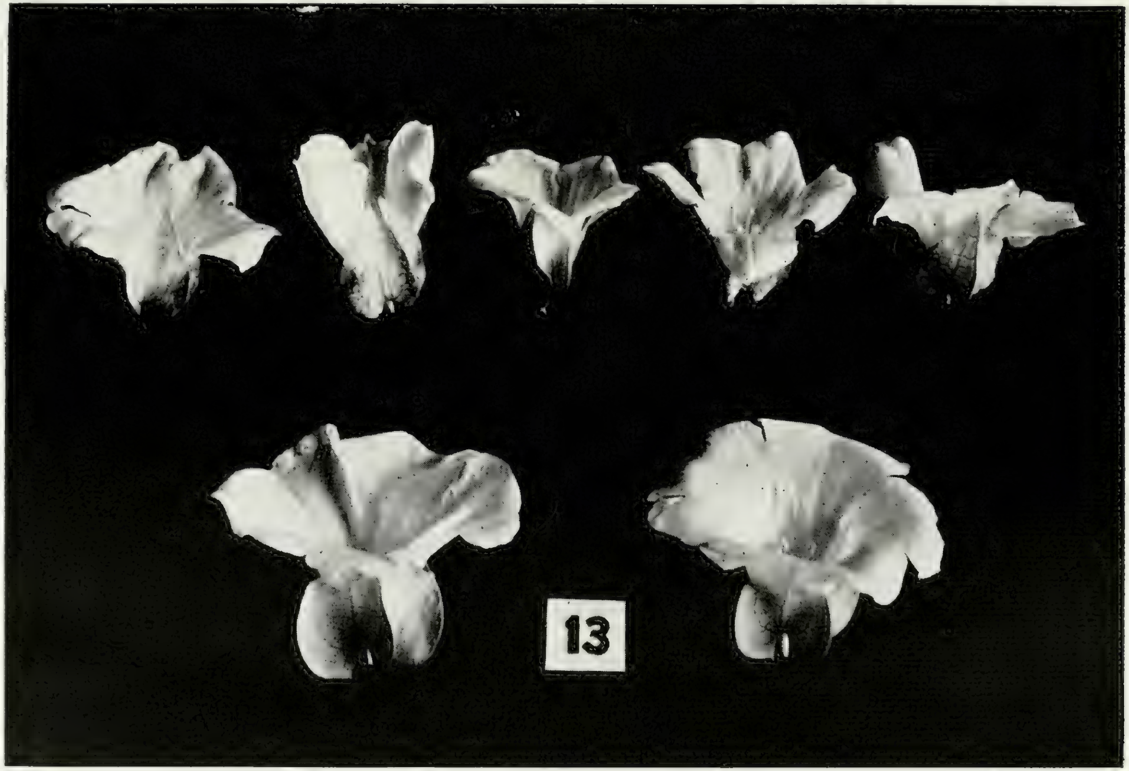
1–3, *Datura Stramonium*: 1, normal leaf; 2, leaf showing mosaic B; 3, leaf showing mosaic C
4–6, *Lycopersicon esculentum*: 4, normal leaf; 5, leaf showing mosaic B; 6, leaf showing mosaic A



NICANDRA PHYSALODES AND PHYSALIS SUBGLABRATA

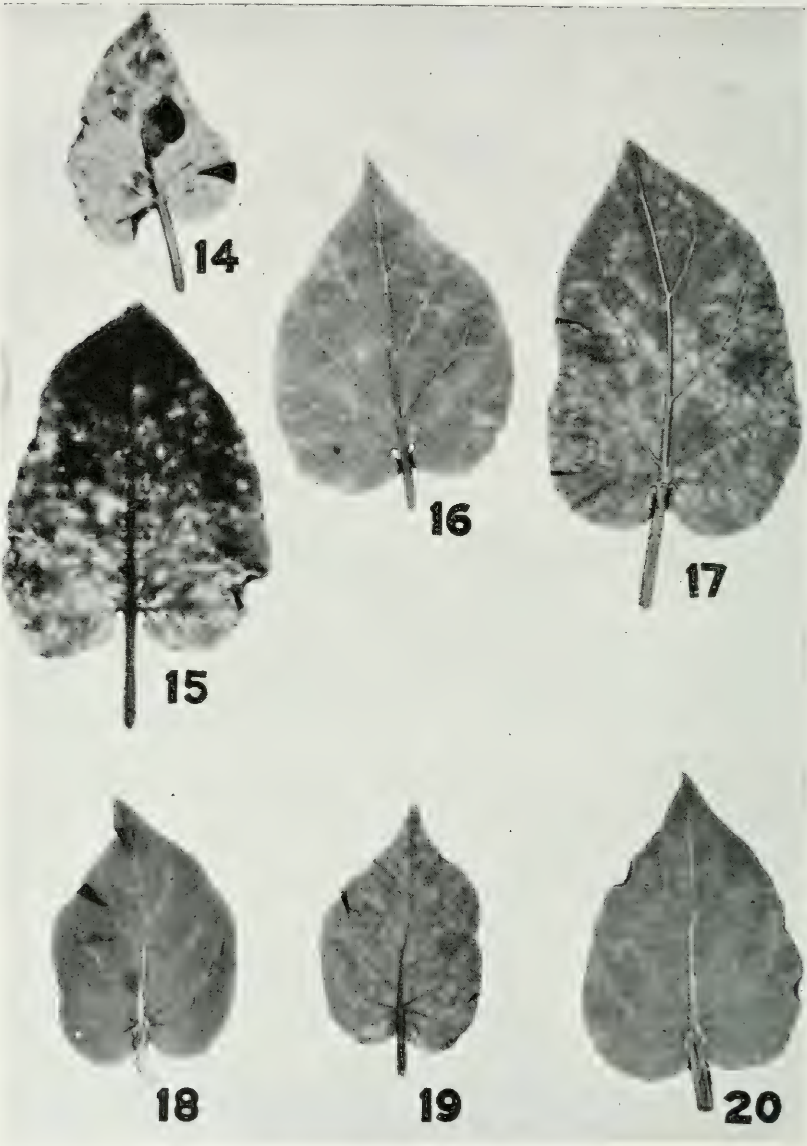
7–11, *Nicandra Physalodes*: 7 and 9, normal leaves; 8 and 11, leaves showing mosaic B; 10, leaf showing mosaic H

12, *Physalis subglabrata*, top of plant, showing mosaic A



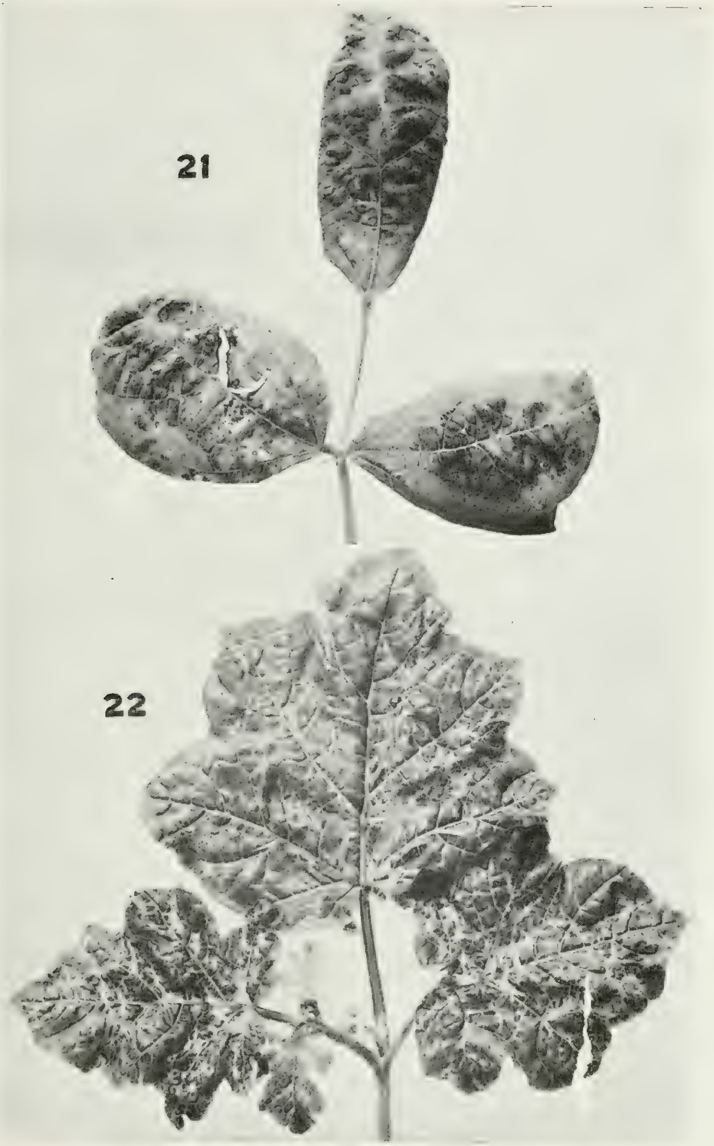
FLOWERS OF NICANDRA PHYSALODES

Flowers in upper row, from plant affected with mosaic H; large flowers below, from normal plant



NICOTIANA GLUTINOSA

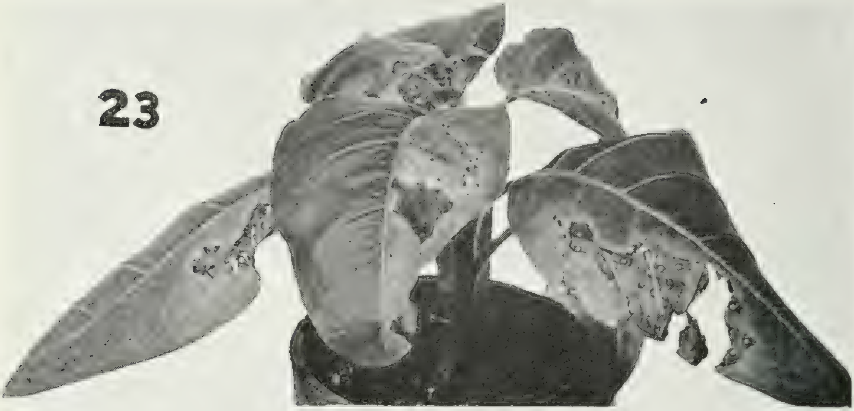
14 and 15, Leaves showing mosaic H; 16 and 20, leaves showing mosaic D; 17 and 19, leaves showing mosaic B; 18, normal leaf



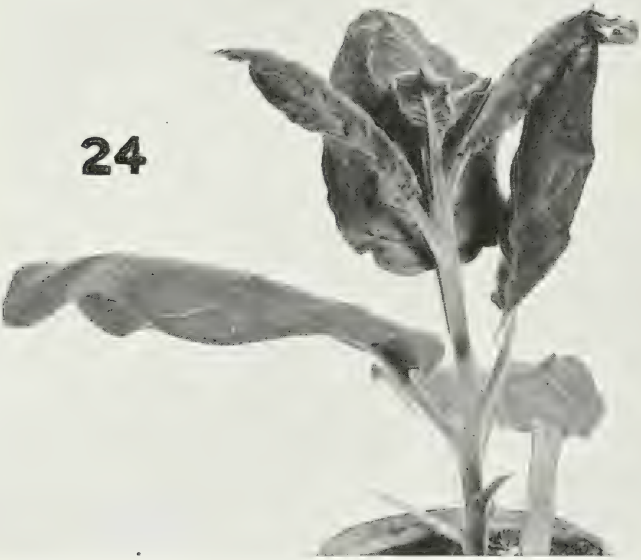
PHASEOLUS VULGARIS AND SOLANUM ACULEATISSIMUM

21, *Phaseolus vulgaris*, leaf showing mosaic G
22, *Solanum aculeatissimum*, leaves showing mosaic A

23



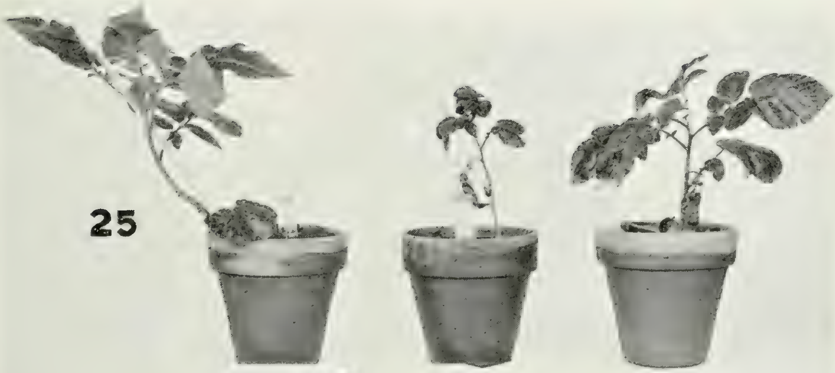
24



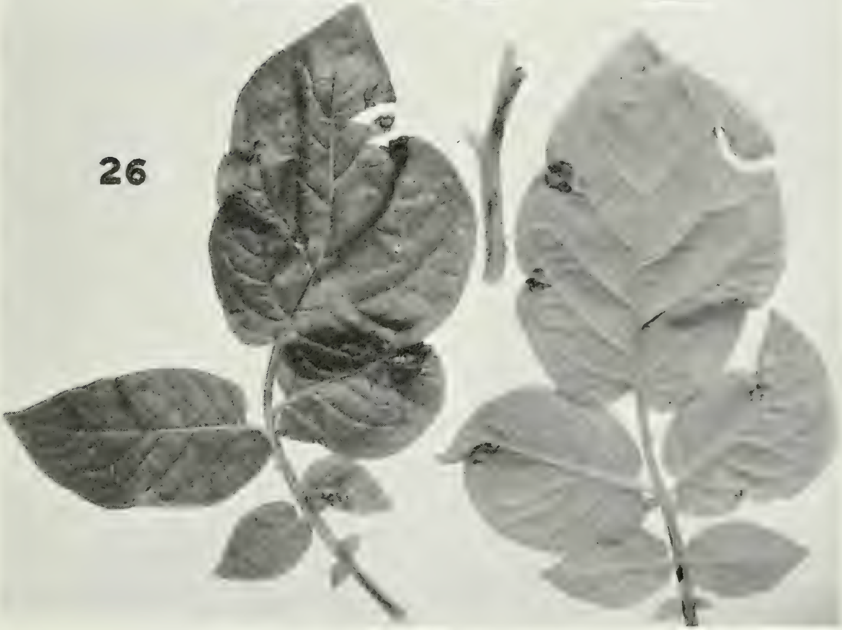
NICOTIANA RUSTICA

23, Plant showing mosaic A; the areas near the points of inoculation, and the youngest leaves, are dead and brown. 24, Normal plant

25



26



SOLANUM TUBEROSUM

25, Plants affected with mosaic A, showing the dropping of the leaves. 26, Leaves, and a part of a stem, from the same plants, showing the puckering around the necrotic spots, and the necrotic streaks on the stem

TABLE 9. INOCULATIONS ON NICOTIANA TABACUM

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A..........	*Lycopersicon esculentum*............	4	4	A
A..........	*Nicotiana Tabacum*................	62	58	A
A..........	*Martynia louisiana*................	3	3	A
A..........	*Solanum aculeatissimum*............	5	4	A
A..........	*Solanum tuberosum*................	30	23	A
B..........	*Lycopersicon esculentum*............	2	1	A
B..........	*Datura Stramonium*................	4	0	....
B..........	*Nicandra Physalodes*..............	4	4	A
B..........	*Nicotiana glutinosa*................	3	0	....
B..........	*Solanum aculeatissimum*............	6	2	A
B..........	*Solanum tuberosum*................	37	1	A
C..........	*Datura Stramonium*................	10	1	A
D..........	*Nicotiana glutinosa*................	6	1	A
E..........	*Phytolacca decandra*..............	9	0	....
F..........	*Rumex obtusifolius*................	3	0	....
G..........	*Phaseolus vulgaris*................	3	0	....
Checks.....	....	46	3	A

* See footnote to table 2, page 13.

TABLE 10. INOCULATIONS ON PHASEOLUS VULGARIS

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A..........	*Nicotiana Tabacum*................	6	0	....
B..........	*Solanum tuberosum*................	6	0	....
C..........	*Datura Stramonium*................	6	0	....
D..........	*Nicotiana glutinosa*................	7	0	....
E..........	*Phytolacca decandra*..............	2	0	....
F..........	*Rumex obtusifolius*................	7	0	....
G..........	*Phaseolus vulgaris*................	12	3	G
H..........	*Echinocystis lobata*................	6	0	....
Checks.....	....	7	0	....

* See footnote to table 2, page 13.

TABLE 11. INOCULATIONS ON PHYSALIS HETEROPHYLLA

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A..........	*Nicotiana Tabacum*................	7	7	A
B..........	*Solanum tuberosum*................	8	0	....
C..........	*Datura Stramonium*................	7	0	....
D..........	*Nicotiana glutinosa*................	8	0	....
E..........	*Phytolacca decandra*................	3	0	....
F..........	*Rumex obtusifolius*................	7	1	A
G..........	*Phaseolus vulgaris*................	3	0	....
H..........	*Echinocystis lobata*................	7	0	....
Checks.....	....	7	0	....

* See footnote to table 2, page 13.

TABLE 12. INOCULATIONS ON PHYSALIS SUBGLABRATA

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A..........	*Lycopersicon esculentum*............	2	2	A
A..........	*Nicotiana Tabacum*................	21	21	A
B..........	*Solanum tuberosum*................	24	3	Uncertain
C..........	*Datura Stramonium*................	21	0	....
D..........	*Nicotiana glutinosa*................	24	0	....
E..........	*Phytolacca decandra*................	21	0	....
F..........	*Rumex obtusifolius*................	21	6	Uncertain
G..........	*Phaseolus vulgaris*................	9	0	....
H..........	*Echinocystis lobata*................	21	0	....
Checks.....	....	23	0	....

* See footnote to table 2, page 13

TABLE 13. INOCULATIONS ON PHYTOLACCA DECANDRA

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Lycopersicon esculentum*	2	0	...
A	*Nicotiana Tabacum*	18	0	...
A	*Solanum aculeatissimum*	2	0	...
A	*Solanum tuberosum*	2	0	...
B	*Datura Stramonium*	2	0	...
B	*Lycopersicon esculentum*	2	0	...
B	*Nicandra Physalodes*	5	0	...
B	*Nicotiana glutinosa*	2	0	...
B	*Solanum aculeatissimum*	2	0	...
B	*Solanum tuberosum*	35	0	...
C	*Datura Stramonium*	16	0	...
D	*Nicotiana glutinosa*	9	0	...
E	*Phytolacca decandra*	17	16	E
F	*Rumex obtusifolius*	14	0	...
G	*Phaseolus vulgaris*	5	0	...
H	*Echinocystis lobata*	5	0	...
H	*Nicandra Physalodes*	6	0	...
Checks	...	23	0	...

* See footnote to table 2, page 13.

TABLE 14. INOCULATIONS ON RUMEX OBTUSIFOLIUS

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Lycopersicon esculentum*	2	0	...
A	*Nicotiana Tabacum*	16	0	...
B	*Datura Stramonium*	2	0	...
B	*Nicotiana glutinosa*	2	0	...
B	*Solanum tuberosum*	19	0	...
C	*Datura Stramonium*	12	0	...
D	*Nicotiana glutinosa*	15	0	...
E	*Phytolacca decandra*	6	0	...
F	*Rumex obtusifolius*	29	13	F
G	*Phaseolus vulgaris*	3	0	...
Checks	...	14	0	...

* See footnote to table 2, page 13.

TABLE 15. INOCULATIONS ON SOLANUM ACULEATISSIMUM

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Lycopersicon esculentum*	6	5	Uncertain
A	*Nicotiana Tabacum*	32	27	3 A 24 Uncertain
A	*Solanum tuberosum*	7	7	Uncertain
B	*Datura Stramonium*	5	3	Uncertain
B	*Lycopersicon esculentum*	2	2	B
B	*Nicandra Physalodes*	2	2	Uncertain
B	*Nicotiana glutinosa*	5	4	Uncertain
B	*Solanum tuberosum*	35	24	Uncertain
C	*Datura Stramonium*	21	2	1 A 1 Uncertain
D	*Nicotiana glutinosa*	11	0	....
E	*Phytolacca decandra*	9	0	....
F	*Rumex obtusifolius*	12	0	....
G	*Phaseolus vulgaris*	8	0	....
H	*Echinocystis lobata*	7	0	....
H	*Nicandra Physalodes*	2	2(?)	H(?)†
Checks	....	29	0	....

* See footnote to table 2, page 13.

† The author was not sure that these plants were affected with mosaic. The symptoms were certainly not those of either mosaic A or mosaic B.

TABLE 16. INOCULATIONS ON SOLANUM ATROPURPUREUM

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Nicotiana Tabacum*	3	3	A
B	*Solanum tuberosum*	3	2	B
C	*Datura Stramonium*	3	0	....
D	*Nicotiana glutinosa*	3	0	....
F	*Rumex obtusifolius*	3	0	....
H	*Echinocystis lobata*	2	0	....
Checks	....	2	0	....

* See footnote to table 2, page 13.

TABLE 17. INOCULATIONS ON SOLANUM CAROLINENSE

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Lycopersicon esculentum*	2	2	A
A	*Nicotiana Tabacum*	7	7	A
B	*Solanum tuberosum*	9	6	B
C	*Datura Stramonium*	7	0	....
D	*Nicotiana glutinosa*	8	0	....
E	*Phytolacca decandra*	2	0	....
F	*Rumex obtusifolius*	7	0	....
G	*Phaseolus vulgaris*	3	0	....
H	*Echinocystis lobata*	6	0	....
Checks	....	9	0	....

* See footnote to table 2, page 13.

TABLE 18. INOCULATIONS ON SOLANUM DULCAMARA

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Lycopersicon esculentum*	2	0	....
A	*Nicotiana Tabacum*	7	0	....
B	*Solanum tuberosum*	9	0	....
C	*Datura Stramonium*	7	0	....
D	*Nicotiana glutinosa*	8	0	....
E	*Phytolacca decandra*	3	0	....
F	*Rumex obtusifolius*	7	0	....
G	*Phaseolus vulgaris*	3	0	....
H	*Echinocystis lobata*	7	0	....
Checks	....	9	0	....

* See footnote to table 2, page 13.

TABLE 19. INOCULATIONS ON SOLANUM NIGRUM

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Nicotiana Tabacum*	9	5	A
B	*Solanum tuberosum*	9	1	A
C	*Datura Stramonium*	7	0	....
D	*Nicotiana glutinosa*	8	0	....
E	*Phytolacca decandra*	3	0	....
F	*Rumex obtusifolius*	7	0	....
G	*Phaseolus vulgaris*	3	0	....
H	*Echinocystis lobata*	7	0	....
Checks	....	9	1	A

* See footnote to table 2, page 13.

TABLE 20. INOCULATIONS ON SOLANUM TUBEROSUM

Mosaic*	Suscept, source of inoculum	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
A	*Lycopersicon esculentum*	26	22	21 A 1 A and B
A	*Nicotiana Tabacum*	14	2	1 A 1 A and B
B	*Lycopersicon esculentum*	11	9	B
B	*Nicandra Physalodes*	9	0	....
B	*Nicotiana glutinosa*	1	0	....
B	*Solanum tuberosum*	53	31	B
C	*Datura Stramonium*	2	0	....
D	*Nicotiana glutinosa*	1	0	....
E	*Phytolacca decandra*	2	0	....
F	*Rumex obtusifolius*	2	0	....
G	*Phaseolus vulgaris*	1	0	....
Checks	Rubbed	6	0	....
	Grafted	9	5	4 A 1 B
	Untreated	23	0	....

* See footnote to table 2, page 13.

TABLE 21. RESULTS OF INOCULATIONS OF VARIOUS SPECIES WITH VARIOUS MOSAICS
(Summary of tables 2 to 20)

Species inoculated	Mosaic	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
Datura meteloides	A	3	0	....
	B	7	5	Uncertain
	C	7	4	Uncertain
	D	7	2	Uncertain
	E	3	0	....
	F	5	0	....
	G	3	0	....
	H	7	0	....
	Checks	7	0	....
Datura Stramonium	A	19	3	C
	B	60	55	41 B 14 Uncertain
	C	28	21	4 B 11 C 3 B and C 3 Uncertain
	D	23	10	9 C 1 Uncertain
	E	10	0	....
	F	14	0	....
	G	9	0	....
	H	13	0	....
	Checks	28	0	....
Lycopersicon esculentum	A	90	87	A
	B	62	46	1 A 32 B 13 Uncertain
	C	9	0	....
	D	7	0	....
	E	3	0	....
	F	8	0	....
	G	3	0	....
	H	7	0	....
	Checks	39	0	....
Martynia louisiana	A	4	4	Uncertain
	B	4	0	....
	D	4	2	Uncertain
	E	5	0	....
	F	4	0	....
	G	4	0	....

TABLE 21 *(continued)*

Species inoculated	Mosaic	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
Nicandra Physalodes	A	35	6	B
	B	149	126	B
	C	15	0	
	D	16	0	
	E	7	0	
	F	11	0	
	G	7	0	
	H	50	18	H
	Checks	65	1	B
Nicotiana glutinosa	A	28	2	1 B 1 Uncertain
	B	62	50	B
	C	26	3	2 B 1 D
	D	37	25	1 B 17 D 7 Uncertain
	E	8	0	
	F	13	0	
	G	7	0	
	H	17	4	H
	Checks	21	0	
Nicotiana rustica	A	32	28	A
	B	23	1	A
	C	12	0	
	D	11	0	
	E	8	0	
	F	11	1	A
	G	6	0	
	H	15	0	
	Checks	23	1	A
Nicotiana Tabacum	A	104	92	A
	B	56	8	A
	C	10	1	A
	D	6	1	A
	E	9	0	
	F	3	0	
	G	3	0	
	Checks	46	3	A

TABLE 21 (*continued*)

Species inoculated	Mosaic	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
Phaseolus vulgaris	A	6	0	
	B	6	0	
	C	6	0	
	D	7	0	
	E	2	0	
	F	7	0	
	G	12	3	G
	H	6	0	
	Checks	7	0	
Physalis heterophylla	A	7	7	A
	B	8	0	
	C	7	0	
	D	8	0	
	E	3	0	
	F	7	1	A
	G	3	0	
	H	7	0	
	Checks	7	0	
Physalis subglabrata	A	23	23	A
	B	24	3	Uncertain
	C	21	0	
	D	24	0	
	E	21	0	
	F	21	6	Uncertain
	G	9	0	
	H	21	0	
	Checks	23	0	
Phytolacca decandra	A	24	0	
	B	48	0	
	C	16	0	
	D	9	0	
	E	17	16	E
	F	14	0	
	G	5	0	
	H	11	0	
	Checks	23	0	
Rumex obtusifolius	A	18	0	
	B	23	0	
	C	12	0	
	D	15	0	
	E	6	0	
	F	29	13	F
	G	3	0	
	Checks	14	0	

TABLE 21 (*continued*)

Species inoculated	Mosaic	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
Solanum aculeatissimum	A	45	39	3 A 36 Uncertain
	B	49	35	2 B 33 Uncertain
	C	21	2	1 A 1 Uncertain
	D	11	0	....
	E	9	0	....
	F	12	0	....
	G	8	0	....
	H	9	2 (?)	H (?)*
	Checks	29	0	....
Solanum atropurpureum	A	3	3	A
	B	3	2	B
	C	3	0	....
	D	3	0	....
	F	3	0	....
	H	2	0	....
	Checks	2	0	....
Solanum carolinense	A	9	9	A
	B	9	6	B
	C	7	0	....
	D	8	0	....
	E	2	0	....
	F	7	0	....
	G	3	0	....
	H	6	0	....
	Checks	9	0	....
Solanum Dulcamara	A	9	0	....
	B	9	0	....
	C	7	0	....
	D	8	0	....
	E	3	0	....
	F	7	0	....
	G	3	0	....
	H	7	0	....
	Checks	9	0	....
Solanum nigrum	A	9	5	A
	B	9	1	A
	C	7	0	....
	D	8	0	....
	E	3	0	....
	F	7	0	....
	G	3	0	....
	H	7	0	....
	Checks	9	1	A

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* The author was not sure that these plants were affected with mosaic. The symptoms were certainly not those of either mosaic A or mosaic B.

TABLE 21 (*concluded*)

Species inoculated	Mosaic	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
Solanum tuberosum	A	40	24	22 A 2 A and B
	B	74	40	B
	C	2	0	
	D	1	0	
	E	2	0	
	F	2	0	
	G	1	0	
	Rubbed checks	6	0	
	Grafted checks	9	5	4 A 1 B
	Untreated checks	23	0	

INTERPRETATION OF RESULTS

Liability to error

Reasonable precautions were taken to prevent errors. In spite of these, there are evidences in the data that errors did occur. Errors in diagnosis were reduced to a minimum by keeping the plants growing vigorously as much as possible, and discarding doubtful cases. It is very likely that some of the species recorded as not susceptible were carriers without showing symptoms. It was not always found possible to record the type of mosaic present. Errors due to accidental contamination did occur, especially during the early part of the experimental work when the hands were applied to the plants in making inoculations. After this practice was discontinued, very little contamination occurred. Some of the earlier experiments were omitted where there was evidence of contamination. Aphids occasionally became fairly abundant in the greenhouses in spite of frequent fumigation, but apparently they did not cause much natural spread. White flies, thrips, and red spiders were sometimes annoying, but there was no indication that they were ever instrumental in transmitting mosaic.

While accidental infections were not frequent, they occasionally did occur. If, therefore, only three or four plants showed positive results from a given transfer, this cannot be taken as proof that the species is susceptible to that particular virus.

Reliability of numerical results

In interpreting the data, it is felt that too much reliance cannot be placed on numerical results. Consideration must be given also to such matters as the conditions under which the particular results were obtained, and the clearness of the symptoms obtained. For example, the author has no hesitation in stating that *Nicotiana glutinosa* is susceptible to mosaic H, although only four positive cases were obtained out of seventeen plants inoculated. The symptoms were so different from those obtained on this suscept when inoculated with mosaic B or mosaic D, that the positive cases could not be regarded as contaminations. However, no such positive statement can be made concerning mosaic C on this plant. It would appear that the three positive cases obtained with this mosaic were contaminations, although this is by no means certain. Taking all such factors into consideration, table 22 has been prepared to show what interpretation is placed on the data by the author. Doubtless, further work will require changes in the conclusions, particularly with reference to negative results.

TABLE 22. SUSCEPTIBILITY OF SPECIES TO MOSAICS

Species inoculated	Mosaic							
	A	B	C	D	E	F	G	H
Datura meteloides	—	+	+	?	—	—	—	—
Datura Stramonium	—	+	+	?	—	—	—	—
Lycopersicon esculentum	+	+	—	—	—	—	—	—
Martynia louisiana	+	—	..	?	—	—	—	..
Nicandra Physalodes	—	+	—	—	—	—	—	+
Nicotiana glutinosa	—	+	—	+	—	—	—	+
Nicotiana rustica	+	—	—	—	—	—	—	—
Nicotiana Tabacum	+	—	—	—	—	—	—	..
Phaseolus vulgaris	—	—	—	—	—	—	+	—
Physalis heterophylla	+	—	—	—	—	—	—	—
Physalis subglabrata	+	?	—	—	—	?	—	—
Phytolacca decandra	—	—	—	—	+	—	—	—
Rumex obtusifolius	—	—	—	—	—	+	—	..
Solanum aculeatissimum	+	+	—	—	—	—	—	?
Solanum atropurpureum	+	+	—	—	..	—	..	—
Solanum carolinense	+	+	—	—	—	—	—	—
Solanum Dulcamara	—	—	—	—	—	—	—	—
Solanum nigrum	+	—	—	—	—	—	—	—
Solanum tuberosum	+	+	—	—	—	—	—	..

Inoculations from potato

In addition to the data presented in tables 2 to 22, some interesting results were obtained by inoculating various plants with material obtained from potato. These results seem to require special consideration. At various times *Nicandra Physalodes* and *Nicotiana glutinosa* plants were inoculated with leaves from potato plants which were affected with mosaic A. Almost invariably these inoculations gave positive results, although it was found impossible to secure infection with mosaic A when material was taken from species other than potato. At first these positive results were considered as evidence of contamination, but the consistency of the results appeared to indicate some other cause. As a check on these results, inoculations were made with supposedly healthy potatoes. Positive results were obtained also in these cases. To check up on this unexpected development, more extensive inoculations were made. More than a hundred tubers of the Green Mountain variety were used, and the freedom of the potatoes from mosaic was checked by planting the tuber remnants in tuber units out of doors. The results were practically always positive. The data on these inoculations are given separately in table 23:

TABLE 23. INOCULATIONS WITH SOLANUM TUBEROSUM

Species inoculated	Type of symptoms on potato	Number of plants inoculated	Number of plants showing symptoms	Type of symptoms observed
Nicandra Physalodes	A	18	16	B
Nicotiana glutinosa	A	8	6	B
Solanum aculeatissimum	A	2	2	*
Datura Stramonium	None	4	4	B
Nicandra Physalodes	None	55	46	B
Nicotiana glutinosa	None	60	59	B

* The plants appeared to be affected with a mixture of mosaics A and B.

These results are strikingly similar to those recently reported by Johnson (1925). Their exact significance is not clear. Two theories have been suggested to account for this phenomenon. According to one theory, the protoplasm of the potato acts as a pathogene and enters into a kind of symbiotic relation with the protoplasm of the new suscept. This theory is attractive because it suggests an explanation of the etiology of mosaic diseases. The alternative theory is that the potatoes used actually carried a virus; that is to say, they had mosaic although they appeared to be healthy. Schultz and Folsom (1923) have shown that potatoes are subject to at least three mosaics which differ in the readiness with which they can be detected. It is well known that even the more pronounced types of potato mosaic may be masked under some field conditions. Is it not reasonable to suppose that there might be a fourth type of potato mosaic so obscure as to be masked even under the most favorable conditions? Such a mosaic would presumably not have much effect on the yield, and, since it could not be detected by ordinary inspection methods, it would not tend to be eliminated, but, on the other hand, would tend to become universal in a tuber-propagated crop such as the potato. Fields of beans are sometimes seen showing an almost 100-per-cent infection, even though bean mosaic is not 100-per-cent seed-transmitted and though bean mosaic materially reduces the yield.

General results

The most significant feature of the experiments is not a determination of species susceptible to any particular virus, but the fact that different mosaics on the same species exhibit symptoms which are sufficiently different to be easily distinguishable. This is not the first time such a phenomenon has been observed, it having been previously reported by Allard (1916), Jagger (1918), Schultz and Folsom (1923), and Johnson (1925). It is rather surprising that such differences in symptoms have not been reported by other authors who have done extensive cross-inoculation work.

CONCLUSIONS

A comparison of the results of cross-inoculation experiments reported in literature reveals many cases in which the results are in conflict. A review of the literature discloses also the fact that most authors have omitted consideration of a factor here shown to be important, namely, the identity of the mosaic concerned. It has been shown here that a single species may be affected by two or more distinct mosaics, which differ not only in their symptoms but also in their suscept range and probably in other characters as well. It may be that this failure to identify the mosaic concerned accounts for many of the discrepancies found in the literature.

As in the case of diseases caused by bacteria, fungi, and so forth, it is found that some viri are able to produce disease on a wide variety of species distributed in different families, and are at the same time unable to infect species in the same genus as their usual suscepts; while other viri seem to be more restricted in their range of suscepts.

SUMMARY

Inoculation experiments were conducted with mosaics on nineteen species of plants, of which fifteen were Solanaceae. All but one of the species tried were shown to be susceptible to one or more of the mosaics used. It appears that the eight mosaics, which for convenience have been designated by letters A to H, are distinct, with the possible exception of two, which may prove to be identical. The differences between the mosaics are shown by the species they are able to attack, and by the different symptoms produced by them when inoculated on the same species. They apparently differ also in the ease of inoculation.

Unusual necrotic symptoms not resembling those generally considered characteristic of mosaics, were produced by mosaic A on *Solanum tuberosum* and *Nicotiana rustica.*

The suscept ranges of the mosaics, in so far as they were determined, were as follows:

Mosaic A : *Lycopersicon esculentum, Martynia louisiana, Nicotiana rustica, N. Tabacum, Physalis heterophylla, P. subglabrata, Solanum aculeatissimum, S. atropurpureum, S. carolinense, S. nigrum, S. tuberosum.*

Mosaic B : *Datura meteloides, D. Stramonium, Lycopersicon esculentum, Nicandra Physalodes, Nicotiana glutinosa, Solanum aculeatissimum, S. atropurpureum, S. carolinense, S. tuberosum.*

Mosaic C : *Datura meteloides, D. Stramonium.*

Mosaic D : *Nicotiana glutinosa.*

Mosaic E : *Phytolacca decandra.*

Mosaic F : *Rumex obtusifolius.*

Mosaic G : *Phaseolus vulgaris.*

Mosaic H : *Echinocystis lobata, Nicandra Physalodes, Nicotiana glutinosa.*

Two mosaics were produced on *Datura meteloides, D. Stramonium, Lycopersicon esculentum, Nicandra Physalodes, Solanum aculeatissimum, S. atropurpureum, S. carolinense,* and *S. tuberosum.* Three mosaics were produced on *Nicotiana glutinosa.*

Some of the data appear to indicate that potatoes which are apparently healthy, carry a virus which is able to produce mosaic symptoms when transferred to other species.

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STUDIES OF THE LIFE HISTORY OF USTILAGO AVENAE (PERS.) JENSEN AND OF USTILAGO LEVIS (KELL. & SWING.) MAGN.

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STUDIES OF THE LIFE HISTORY OF USTILAGO AVENAE (PERS.) JENSEN AND OF USTILAGO LEVIS (KELL. & SWING.) MAGN.[1]

George Raymond Gage

Although seed treatment for the control of the two smuts of oats caused by *Ustilago avenae* (Pers.) Jensen and *U. levis* (Kell. & Swing.) Magn. has been developed to such a point that it is generally regarded as fairly successful, it seems that the ultimate and most economical control of these smuts should lie in the selection or the breeding of resistant varieties. That this is the opinion of most cereal pathologists is evidenced by the marked activity along this line in recent years, as shown by the work of Zavitz (1909, 1914, 1915), Heald (1919), Stapledon (1920), Reed and others (1920, 1924, and 1925), Zade (1922), Arland (1924), Roesch (1926), and many others. Successful procedure toward such an end necessitates not only a thorough knowledge of the physiology and anatomy of the suscept, but also a rather detailed and exact understanding of the life histories of the pathogenes. If one is to test varieties for susceptibility or resistance, he must, above all, understand and be able to manipulate those factors which condition infection.

Because of the many conflicting ideas of various investigators, and especially because of the recent pronouncements on blossom infection made by Zade and his students, it has seemed advisable to reinvestigate the life histories of the pathogenes causing the smut diseases of oats. It is very evident that the life histories of these pathogenes—*Ustilago avenae*, the rough-spored species causing the so-called "loose smut," and *U. levis*, the smooth-spored species causing the so-called "covered smut"—are not adequately understood.

It is not the intention of the writer to bring together into an historical account the development of the present-day ideas on the life histories of these two pathogenes. It will be noticed at once by one who delves into the history of the subject that the writings of the earlier investigators are very confusing and indefinite. All of the loose smuts of cereals were at first considered to be caused by one and the same pathogene, commonly called *Ustilago carbo* or *U. segetum*. It was not until 1888 that

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[1] Also presented to the Faculty of the Graduate School of Cornell University, August, 1926, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

Author's acknowledgment. The investigations discussed in this paper were undertaken at the suggestion of Professor H. H. Whetzel, under whose direction and kindly criticism they were conducted and completed; for all of which, the writer wishes to express his most sincere appreciation.

Jensen proved, by infection experiments, that each suscept—oats, wheat, and barley—had its respective and specific loose-smut pathogene. To the smut fungi on oats he finally gave the name *Ustilago avenae.* He did not, however, at this time distinguish between the two kinds of oat smut. This distinction was made by Kellerman and Swingle (1890). They recognized the covered-smut pathogene to be distinct from that of the loose smut, and gave to it the name *Ustilago avenae,* var. *levis,* which was later raised to specific rank by Magnus.

For a brief but rather comprehensive sketch of this early history, one may consult Fischer von Waldheim (1869–70), Kellerman and Swingle (1890), and McAlpine (1910).

With the exception of the work of Lang (1913), which is discussed later, nothing of very great significance was added to the general fund of information and ideas from 1890 to 1922. During that period the results and conclusions of previous investigators were generally accepted, probably due to the fact that attention was being drawn more toward those diseases for which a satisfactory control had not been discovered.

PRE-ZADIAN CONCEPTIONS

Since the work of Zade has thrown new light upon the subject, which, in the writer's opinion, may be considered a turning point in the knowledge of the life history of *Ustilago avenae,* it may be well to coin the phrase *pre-Zadian conceptions* and under this as a heading recapitulate the status of the ideas on the subject up to 1922. Elaboration of details is limited here, only those essentials being presented which will serve to bring to memory the generally accepted conception of the life histories of these smut fungi. It will be evident that there are several conflicting ideas and that much of the earlier experimental procedure in obtaining data has not been in keeping with the course of events as they occur in nature, and, above all, that many of the conclusions are based on mere assumption.

LIFE HISTORY OF USTILAGO AVENAE

Inoculation

The source of inoculum is generally admitted to be the smutted plant in the field at flowering time. The dark sooty masses of chlamydospores in the diseased heads are disseminated by the wind and come in contact with the flowers of the healthy plant. It was held by Jensen (1888: 400–401) that only those spores lodging within the glumes of the healthy blossoms, which are open for the purpose of pollination, can play a significant rôle in infection. This was later confirmed by the work of Clinton (1900: 308–309), and is borne out also by the fact that glumeless oats seem to suffer more than do glumed varieties. This, however, has been explained by Reed (1920: 38) and others as being due to a more marked susceptibility of the glumeless oats.

Incubation

The incubation stage is held to be largely one of inactivity, extending over the period from blossoming time until shortly after the oats have been sown in the spring, the seed of the suscept and the spore remaining dormant together throughout the winter in storage. The spores as well as the oat seed may remain viable for a long time if kept dry. According to McAlpine (1910: 104), Von Liebenberg germinated the chlamydospores at the end of seven and one-half years.

The active stage of incubation begins shortly after the oats have been sown. Moisture from the soil is absorbed by the seed and the spores alike, and if temperature conditions are favorable they both germinate. Kühn (1858: 48–49) experimented with wheat and the stinking-smut pathogene, and came to the conclusion that penetration took place in the vicinity of the root node. Wolff (1873: 660–661) was of the opinion that penetration could take place only through the young leaf sheath in the case of *Urocystis occulta* on rye. Kühn (1874: 5–6) extended his experiments and concluded that penetration of *Ustilago carbo* (the name then used for the pathogenes causing both the loose smut of barley and that of oats) could take place in the root node, in the first stem node, and also in the internode between the two. Brefeld (1895: 24–26), from spraying germinating oats with sporidia grown in artificial media, concluded that penetration of the axis of the young seedling is effected only in very young stages, and that infection does not result when the seedlings are thus inoculated after the inner leaves have pushed one centimeter through the leaf sheath. Lang (1913: 177–178), working with serial sections, claims the infection court to be the mesocotyl. This, he says, elongates to push the young seedling out of the glumes. The spores germinate for the most part into germ tubes, but sporidia also are sometimes formed. At the end of seven days the mesocotyl is about one-half centimeter long. While the cells are still normally active the germ tubes remain on its surface, but when they begin to die (the mesocotyl being a short-lived structure) the germ tubes penetrate the dying cells on the surface and reach the healthy tissue beneath.

Infection

According to Brefeld (1895: 32–37), the penetrating hyphae enter the seedling axis and grow directly through the tissues and reach the growing apex of the suscept. According to Lang (1913: 178–179), the germ tubes, upon reaching the living cells inside of the dying mesocotyl, turn upward and grow between the cells, obtaining their food by means of haustoria. They traverse the primary node through an opening between the vascular bundle tips and the insertion of the first leaf. Here they branch and are thus established in the growing apex of the suscept. After the tissues of the primary node have become mature, the pathogene is not able to pass through the node and reach the meristem above. The mycelium originating from the hyphae that have successfully passed through the primary node, keeps pace with the growing point as this is pushed upward. The fungus causes a mild stimulation of the suscept cells, which accounts for the slightly more vigorous growth of the infected plants often observed during the period of optimum vegetation.

According to Butler (1918: 179–180), the growing point may sometimes grow away from the pathogene, and the mycelium thus left behind becomes disorganized, breaks up into segments, and disappears in the older tissues, just as it does even in the culms in which the pathogene succeeds in maintaining itself in the growing point.

As the culms elongate, the pathogene accompanying the growing point at last reaches the developing flowers. Here the mycelium branches profusely, becomes swollen and knobbed, and absorbs the differentiating suscept tissues. The chlamydospores are formed inside the terminal branches of the mycelium, the walls of which at length gelatinize and disappear. Thus, in place of the normal flower with its glumes, there is produced a mass of black or dark brown spores ready for dissemination. The degree to which normal tissues are replaced varies, sometimes both the flowers and the glumes being completely replaced, sometimes the glumes remaining partly or entirely intact. It is commonly observed also that only a part of the panicle is smutted, in which case it is always the lower part that is affected.

LIFE HISTORY OF USTILAGO LEVIS

The life history of *Ustilago levis* is held to be very similar to that of *U. avenae*. Much of this similarity has been assumed. The difference in appearance of plants smutted by *U. levis* and by *U. avenae* was brought out by Kellerman and Swingle (1890) when they distinguished between the two pathogenes. With *U. levis*, the heads of the oat plant are not so completely destroyed. The glumes remain intact and it is often difficult to know whether the spores are present without breaking open the glumes. Clinton (1900: 297) says, "These enveloping parts furnish such protection against dissemination of the spores that frequently the smutted flowers may be found in badly smutted grain after it is threshed." He states also that experiments with covered smut show that it is the spores which in the field have succeeded in falling between the open glumes at flowering time, that are blamable for infection.

Lutman (1910: 1200-1206), who produced artificial inoculation by spreading cultures of sporidia on young seedlings, emphasizes the fact that haustoria are absent, the pathogene being entirely intercellular. His description of spore formation is similar to that given above for *U. avenae.*

EPIPHYTOLOGY

Environmental factors governing both loose and covered smut have been assumed to be more or less alike.

Temperature

Low temperatures of the soil are held to favor both pathogenes. Spores germinate readily at temperatures as low as 10° C. At such a temperature oats germinate very slowly. Thus the pathogenes are able to penetrate and reach the growing point of the suscept before it emerges from the soil. If temperatures of 15° or above obtain, then the suscept grows so rapidly that the pathogene has little chance to become established in the tissues of the growing point. This idea was advanced by Brefeld (1895: 25), who, in all of his inoculation experiments, after spraying the young seedlings with sporidia kept them at a temperature of 10° for a period of several days before planting. Clinton (1900: 305) showed by experiment that late planting of oats reduces infection. Lutman (1910: 1201), working with *U. levis*, obtained infected plants by smearing sporidia on the young leaf sheath and keeping the inoculated seedlings at a temperature of 12° for from five to seven days. Heald (1919: 31), however, is of the opinion that late seedings produce more infection. Butler (1918: 180) also takes an opposing view with respect to temperature. He says, "When the temperature at sowing time is low, oats are more likely to escape smut, since the spores require a considerably higher temperature for germinating than the oat grains themselves." Tubeuf (1897: 286–287) clearly has misinterpreted Brefeld when he says Brefeld "found that oat-smut germinated best at 10° C., and not so well above 15° C." Brefeld did not find that the smut spores germinated best at 10° and not so well above 15°, but that effective penetration took place better at 10°.

On the one hand, then, Brefeld, Clinton, and Lutman believe that low temperatures favor infection, while Heald and Butler seem to think that high temperatures are favorable.

Moisture

Moisture relations may be particularly influential at two different periods in the life history of the pathogene—at the time of spore dissemination, and at the time of spore germination. It is obvious that a sufficient supply of moisture is necessary at the time of germination of the seed and the spore. Clinton (1900: 308) is of the opinion that varying amounts of moisture may have a direct effect upon the amount of infection. He experimented with oats sown at different depths, and found that those broadcast resulted in 2 per cent of smut, those planted 1 inch deep resulted in 6 per cent of smut, and those planted 4 inches deep resulted in 10 per cent of smut. This he explains as being due in part to differences in soil-moisture content at the different depths.

At the time of spore dissemination, moisture plays an important part, for it is evident that heavy rains at this time will carry a large number of the spores into the soil and thus decrease the chance of inoculation. On the other hand, rain may aid in lodging the spores between the glumes. Jensen (1888: 405) noted that oats dipped in spore-charged water gave 29 per cent of smut, while those dusted with dry spores resulted in no infection. From this it would seem logical to assume that rains might tend to wash spores from the exterior of the glumes in between the closed glumes and the caryopsis, especially if the rains were light and came after a day favorable for spore dissemination.

THE LEIPZIG INVESTIGATIONS

ZADE'S STUDIES, 1922

As has already been intimated, some of Zade's (1922) observations and conclusions are not in keeping with the conception of the life history of *Ustilago avenae* as it has just been presented.

First of all, Zade confirmed the work of previous investigators in so far as his experiments showed that spores applied to the exterior of the glumes did not produce any appreciable amount of smut. He thinks, however, that the spores which lodge on the kernels of those oats that are deglumed during threshing, play a part in the usual percentage of infection. Spores applied after the glumes were removed gave much better results, but even here a maximum infection of only 26.9 per cent was obtained. Zade concluded that, although spores which lodge between the glumes can cause infection, the degree of infection is not sufficient to explain the frequency of epiphytotic attacks of the disease.

When he applied spores to the blossoms of oats at pollination time and subsequently examined them, *Zade discovered that almost all of the spores which fell on the stigma began to germinate at once* (in from fifteen to twenty hours, in the mild damp weather at the time of his experiment). The promycelia formed from the spores tended to be unusually long, but were otherwise normal and gave rise to budding sporidia. These sporidia produced hyphae which came in contact with the inner epidermis of the glumes and formed mycelium in the parenchyma beneath this epidermis. Zade obtained no evidence of flower infection such as is known in *U. tritici*, the embryo remaining free from invasion and only the glumes being attacked. In fact, he says that *the mycelium does not penetrate the ovary and in no case can be found in, on, or under the epidermis of it.* He believes that the mycelium in the glumes is the most important source of inoculum for seedling invasion, although overwintering spores, or perhaps sporidia, may play a small part.

ARLAND'S STUDIES, 1924

The investigations of Zade were turned over to Arland (1924) for further prosecution. In the main Arland's results confirm those of Zade. He also inoculated oat blossoms at pollination time and noted that the spores germinated in the flower. This took place, however, in four days instead of from fifteen to twenty hours as had been observed by Zade. Moreover, the promycelia did not give rise to sporidia, the temperature at the time being above 30° C. This is in keeping with the recent observations of Bartholomew and Jones (1923 : 575) and of Jones (1923 : 590), who found 30° C. to be the maximum for sporidial production. At lower temperatures Arland noticed sporidial production in a few cases. Zade had suggested that resting sporidia might play a rôle in seedling infection, but Arland refutes this possibility since he was unable to keep dried cultures of sporidia viable for a longer period than six weeks. The ungerminated spores, however, do play an important rôle, in Arland's opinion, since he was able to get fairly high percentages of infection in certain variety tests when he introduced spores between the glumes of mature oats prior to sowing. He detected the development of mycelium not only in the glumes but also in the remains of the stamens and the stigma. *Incidentally he mentions that he found it in a few cases in the epidermis of the ovary.* By a more extensive study of the mycelium he found that it often broke up into biscuit- or dumbbell-shaped pieces which he called *gemmae*. He concludes that the seat of overwintering mycelium *is seldom the epidermis of the pericarp,* but is mostly the glume parenchyma and, without exception, the remains of the stamens and the stigma.

With naked oats which he dusted with spores at blossom time, Arland found that the mycelium which was produced wound itself among the hairs of the caryopsis. *It was seldom noticed in the epidermal cells.* He made variety tests with seed prepared in three ways: first, spores were placed between the glume and the kernel prior to the sowing of the oats; secondly, deglumed kernels were dusted with spores; and thirdly, sporidia were introduced between the glume and the kernel. The average percentage of infection resulting in the first test was 29 per cent, in the second 24.2 per cent, and in the third 5.1 per cent. *Avena nuda,* var. *chinensis,* the glumeless oat used, proved to be much more susceptible than the glumed varieties. *A. strigosa* and *A. brevis* were immune.

In tests on the comparison of smutting in plants produced from the inner grains of the spikelet with that in plants produced from the outer grains, Arland noted that the inner grains produced from three to four times, and in some cases from ten to twenty times, as many smutted plants as did the outer and larger grains.

ZADE'S STUDIES, 1924

Zade (1924) published a second paper which is a revised statement based on the investigations published by Arland. It appears that Arland, and also Diehl and Roesch, who published their theses later, were students working under Zade. Reference is made by the last two men to an unpublished thesis by Neumeyer, and, since Zade remarks that the blossom-inoculation work was carried out at his suggestion by Neumeyer, it is assumed that his paper (which the writer has not seen) covers in general the same investigations as are discussed in Zade's first paper (1922).

Since the foregoing account of Arland's studies covers all of the essentials given in the second paper by Zade, that paper is not discussed here in detail. It may be well, however, to mention the conclusions which Zade drew at that time. These were as follows:

The seat of the resting mycelium may be: (1) the parenchyma of the glumes; (2) *the epidermis of the pericarp, where the mycelium is very weak and only superficially or seldom present*; (3) in naked oats, the hairs of the caryopsis, where the mycelium is interwoven with them; (4) the remains of the anthers and the stigma, without exception; and (5) the remains of the lodicules, only seldom.

Zade believes that, in the case of naked oats, there is no possibility other than that infection of the seedling results from spores, and also mycelium and gemmae, which are on the epidermis of the caryopsis. This, he says, remains to be investigated. He states that he has suggested to Roesch (whose work is considered later) a method of inoculating glumes which will prove whether the resting mycelium and the gemmae are or are not actually the true inoculum for seedling invasion. So far, he says, this has only been assumed.

DIEHL'S STUDIES, 1925

Diehl (1925), continuing the work on oat smut and following in general the methods of Zade and Arland, came to the following conclusion:

Germination of the spores takes place most promptly on the moist stigma. If the weather is wet and cool, sporidia are produced; if it is warm and dry, the spores germinate directly into germ tubes. However, if the moisture within the flowers is sufficient, the spores may germinate even if they do not come in contact with the stigma. Only in exceptional cases—that is, in very dry years—do the spores on the ovary remain ungerminated. In these cases, however, they can play a certain part in infection, even though a subordinate one.

In addition to finding mycelium in the glumes and the stigma and anther remains, Diehl found it in the lodicules and also observed that *it was very abundant in the epidermis of the ovary.* In the case of the glumes and the epidermis of the caryopsis, long hyphae were produced. Gemmae were formed in the stigma and anther remains, both from mycelium and from sporidia. In the latter case the sporidia merely enlarged and took on heavier walls.

Diehl noticed also a considerable difference in the manner in which blossoms opened for pollination. The blossoms of several varieties with which he worked did not open at all.

For the purpose of making tests of various disinfectants, Diehl removed oat glumes, sterilized them, and inoculated them with spores, thereby producing mycelium in them. He then returned these glumes to the kernels. In a like manner, he inoculated and brought about invasion of stamen remains and introduced these between the glumes. It is evident from this that he does not consider the mycelium in the pericarp as being very important.

ROESCH'S STUDIES. 1926

Roesch (1926), the latest of the Leipzig investigators cooperating on the studies on oat smut, pursued methods similar to those of his coworkers. His results and conclusions are as follows:

On the stigma, 100-per-cent germination of spores may take place. *On the epidermis of the caryopsis, the spores germinate very slowly and give rise to only a few short hyphae.* Ungerminated spores may be found occasionally on the epidermis and the epidermal hairs of the caryopsis. These do not play any significant rôle in infection, the resting mycelium and the gemmae on and in the remains of the stigma and the stamens, in the glumes, in the caryopsis epidermis, and in the lodicules, being concerned in the invasion of the seedling.

With naked oats the invasion of the seedling takes place only by the resting mycelium on the caryopsis epidermis, by gemmae, and, in isolated cases, by overwintered spores. Additional infection may also result from spores which reach the oats at threshing time and which germinate the next spring. Roesch considers that the pieces of stigma and anther remains with mycelium and gemmae, which he found between the epidermal hairs of naked oats, can play no important rôle in infection, since the hairs are broken off to a great extent during threshing.

Roesch made studies of seedling invasion in the following manner: Spores were germinated in a decoction of oat glumes. When there was an accumulation of mycelium on the surface of the cultures, young seedlings produced from deglumed seed were dipped into the culture solution and thereby the sprouts and kernels were inoculated with mycelium. Roesch describes the invasion of the seedling in some detail. The hyphae penetrate the epidermis and the outer layers of cells by boring directly through them. In the inner tissues, however, the mycelium becomes intercellular. The protoplasm of the invading hyphae migrates with the hyphal tips and leaves empty walls behind in its path. Penetration was observed in the axis as well as in the coleoptile. In the latter, however, the penetrating hyphae, in most cases, did not enter as far as the third leaf. To determine in what stage of development the seedling is most susceptible, Roesch inoculated seedlings of different lengths, varying from 3 to 4 millimeters to 3 centimeters. After a period of three days, many relatively long hyphae were found in the younger seedlings while in the older ones hyphae were very scarce. The fungus seems to have the power to dissolve only the cellulose of thin cell walls. The susceptible stage of the young seedling expires when the first leaf breaks out of the coleoptile.

The pathogene can penetrate such fully immune varieties of oats as *Avena brevis.* In these cases the hyphae grow very slowly and produce plasma-poor threads, possibly as a result of unfavorable growth conditions. The pathogene may also penetrate even the seedlings of wheat, barley, rye, and oat grass. Further development of the hyphae, however, does not take place in these plants. With peas and beets, no penetration was observed.

In testing the same varieties during two different years, Roesch obtained no great differences in percentages of infection for the respective varieties, and therefore he concludes that susceptibility or resistance is an hereditary variety character.

By sowing some of his oats later in the season he noted higher amounts of infection. He advises, therefore, early spring sowing as a means of control.

The smaller inner grains produced higher amounts of smut, just as had been noted by Arland. Roesch believes this is due to the lower vitality of the inner grains, for he noticed that the plants developing therefrom grew more slowly in their early stages.

In several varieties of oats, Roesch also, as had Diehl, noticed considerable variations in the degree to which the blossoms opened for pollination. He concludes, therefore, that the possibility of inoculation is thus affected. Even in the same spikelet the second and the third flower open wider, and for a longer time, than the first flower. On this account Roesch recommends that the small grains be eliminated from the seed.

For the purpose of testing varieties, Roesch placed pieces of glumes in petri dishes and inoculated them with spores. When mycelium had developed he inserted these pieces of glumes between the glumes of his seed oats. This was the method previously mentioned as being suggested by Zade. The highest percentage of infection resulting from the oats which were inoculated in this way was 26.4 per cent. In most of the varieties tested the average percentage of resulting smutted plants was less than 6 per cent.

COMMENTS ON THE LEIPZIG INVESTIGATIONS

The most outstanding contribution to our knowledge of the life history of *Ustilago avenae* since the pioneer investigations of Brefeld, Jensen, and Kellerman and Swingle, at least in the writer's opinion, has been this discovery of spore germination at blossoming time by Zade and his students. Brefeld's successful but artificial method of spraying young seedlings with sporidia grown in artificial culture was accepted as final confirmation of the conclusions of Kühn and Wolff in regard to the primary nature of seedling infection. The recent discovery of immediate spore germination has opened again what has been considered a closed chapter, and has especially stimulated the writer to seek further enlightenment on the life histories of the oat-smut pathogenes.

It may be recalled that Zade, Neumeyer, Arland, Diehl, and Roesch worked only with *U. avenae.* The life history of *U. levis* also needs investigation. Furthermore, it may be concluded that these German workers consider the mycelium and the gemmae in the glumes, and possibly in the stigma and anther remains, to be the chief inoculum for seedling invasion. Certainly they do not consider the mycelium in the epidermis of the caryopsis pericarp as being especially significant. Zade stated in his first paper (1922) that he would undertake to sow oats from artificially inoculated blossoms, of which part would be glumed and part deglumed. If his assumptions were correct, infected oat plants should come from the glumed oats, while the deglumed oats should give rise to healthy plants. So far this has not been reported as carried out. In his second paper, it will be remembered, he says that he suggested a method to Roesch whereby the glumes of oats might be inoculated and become infected. This method has been described in the discussion of Roesch's studies. Zade predicted that the stands of plants coming from such inoculated seed would show whether the resting mycelium and the gemmae in the glumes were really the true inoculum for seedling invasion, a theory which thus far had only been assumed. It will also be remembered that Roesch's results show a maximum percentage of 26.4 per cent of smutted plants, which was an exception to the general average of less than 6 per cent. In Zade's first paper he states that the 26.9 per cent of smut obtained by dusting spores on deglumed kernels is insufficient to explain the epiphytotic attacks often occurring. If this is true, the 26.4 per cent obtained by Roesch offers no better explanation. This, it seems to the writer, throws some doubt on the possibility that the mycelium and the gemmae in the glumes play an important rôle.

THE CORNELL INVESTIGATIONS

BLOSSOM INFECTION AND SEEDLING INVASION

Before proceeding with a detailed discussion of the investigations carried on at Cornell University, it may be well to indicate the principal problem about which these researches center. This may be presented in brief by contrasting the conclusions of Zade and his colleagues with that of the writer on the point at issue.

On microscopic examination of blossoms which were artificially inoculated with spores of *Ustilago avenae* at pollination time, it was observed that the majority of the spores had germinated. No sporidia were produced, probably due to unfavorable temperature as suggested by the investigations of Arland (1924: 80), Bartholomew and Jones (1923: 575), and Jones (1923: 590). By frequent examination for several weeks after germination of the spores, it was found that mycelium is developed which penetrates and becomes established in the glumes (Plate II, 3), just as the German workers found. *On the other hand, mycelium was also found abundantly on, in, and under the epidermis of the caryopsis* (Plate I, 2). The presence of abundant mycelium in the pericarp is reported also by Diehl, as previously pointed out. Zade was unable to find such mycelium in any instance, and Arland noticed it only in a few cases. Roesch does not make particular mention of it. All of these investigators assume that the mycelium and the gemmae in the glumes and in the anther and stigma remains are the chief sources of inoculum for invasion of the developing seedling. *In the opinion of the writer, however, it is the mycelium developed on, in, and under the epidermis of the caryopsis that is accountable for most of the seedling invasion.* The data on which his conclusions as to the character of blossom infection and subsequent seedling invasion are based, are presented in the following pages.

Positional relation of pathogene structures to the oat flower and grain

Investigations by the writer were begun in June, 1924. It was recognized at once that accurate interpretation of the data would depend to a very great extent upon the ability to work with material (oats for seeding) the exact history of which was definitely known. Since no such seed was available, several preliminary studies were made with a supply of oats which had been harvested the previous year from a field known to have had considerable smut in it.

The most logical way to proceed seemed to be to determine first the fate of the chlamydospores introduced into the blossoms at flowering time, or, rather, the position in or on the seed of the structures of the pathogene which live over from blossoming time to planting. This was done by a process of elimination.

Five similar lots of seed (the term *seed* is used to designate the caryopsis with its enveloping glumes) from the supply mentioned above, were used. The first was treated with formalin; the second was untreated; the third was deglumed (the glumes being removed carefully with forceps); the fourth was deglumed and then about one-fourth of the stigma end of the caryopsis was cut off; and in the fifth, one-fourth of the stigma end of the entire englumed seed was removed. All five lots were sown at the same time, June 10, at the rate of 13 grams to a 15-foot row and approximately 1½ inches deep. The results are given in table 1:

TABLE 1. Percentage of Smutted Plants Resulting from Different Treatments

Treatment	Number of plants	Number smutted	Per cent smutted	Per cent of smut presumably due to parts removed
Formalin	600	0	0	
Untreated	600	40	6.67	
Glumes removed	692	28	4.05	2.62
Glumes and stigma end removed	486	16	3.29	3.38
Glume tips and stigma end removed	608	34	5.59	1.08

Since 2.62 is 39.28 per cent of 6.67 and may be assumed to represent the percentage of total infection due to parts of the pathogene in or on the glumes, then the remaining 60.72 per cent must necessarily come from pathogene structures in or on the caryopsis. Regardless of the nature of these parts—that is, whether they are spores or mycelium—*the fact that they were accountable for 60.72 per cent of all infected plants would indicate at once that mycelium in the glumes is not the chief cause of seedling invasion.* By subtracting the percentage due to pathogene structures in or on the glumes from the percentage due to those in or on the glumes and the stigma end combined, the amount due to pathogene structures in or on the stigma end alone may be obtained, namely, 11.4 per cent. This indicates that those pathogene structures in or on the

caryopsis which would account for 60.72 per cent of the infected plants. are not necessarily restricted to the stigma end.

These preliminary studies gave data which indicate only the position of the pathogene organs which are accountable for seedling invasion. In order to determine (1) the nature of these structures—that is, whether they are mycelium or spores—and (2) whether they are external to the caryopsis or within its tissues, more exacting experiments were undertaken.

Nature and relative importance of the various pathogene structures in the oat flower and grain

Scources of, and methods of selecting, experimental materials

Ustilago avenae.—As has already been stated, the writer considered it absolutely necessary to have seed of known history to work with. While the foregoing preliminary studies were being carried out, such seed was obtained in the following manner:

Supplies of Swedish Select, a glumed variety of oats, and Selection 202, a glumeless variety, were obtained from the Department of Plant Breeding at Cornell University. The Swedish Select oats were known to have come from an infested field. The lot of the glumed variety was divided into two parts, one of which was untreated and sown in plots at East Ithaca in order to guarantee a supply of spores for inoculation purposes. The other part, and the supply of the glumeless variety, were treated with formalin and sown in plots near Forest Home, which is about a mile from East Ithaca. These were to be used for producing plants for inoculation.

Shortly before the panicles emerged from the leaf sheaths of the plant at Forest Home, some of both varieties were covered with wax-paper bags to insure a supply of each kind against natural or accidental inoculation. They were harvested later, bags and all. Thus, clean uninoculated seed was obtained.

At blossoming time spores of *U. avenae* were obtained from the untreated plots at East Ithaca. These were examined microscopically to make sure of their rough-spored character, since covered smut also was present in the plots. The so-called ''loose'' character of the lesions caused by *U. avenae* does not seem to be consistent, since gradations may be found that correspond more to the ''covered'' character of the *U. levis* lesion.

When the blossoms in the plots at Forest Home were fully developed and undergoing pollination, some of both varieties were artificially inoculated. This was accomplished much after the method employed by Zade, namely, by tapping a small camel's-hair brush, previously dipped in spores, against the finger, thus introducing spore material into the blossoms without touching any of the floral parts. Inoculated blossoms were tagged by means of colored strings. Flowers in a spikelet, not mature at the time of dusting, were removed with a pair of scissors.

Three days after dusting, flowers of each variety were examined microscopically. As has previously been stated, the majority of the spores had germinated. Those on the stigma seemed to have germinated first (Plate I, 1)—probably due to the sugary excretion as Zade has suggested; but, in addition, the spores lodged on the ovary walls were also fully germinated at the end of five days. This took place in both the glumed and the glumeless varieties. In no case, however, was sporidial production observed. The average temperature recorded by the United States Weather Bureau Station at Ithaca during this period was slightly under 30° C.

At the end of twelve days mycelium could be detected in the glumes and in the pericarp epidermis in both varieties. For detection of the mycelium the writer used at first an aqueous solution of eosin, but later, on becoming acquainted with a stain known as "cotton blue,"[2] he found it an easy matter to demonstrate the mycelium in both the glume parenchyma and the pericarp epidermis. This stain was used in all subsequent mycelial studies.

When mature, the inoculated oats were harvested and stored in paper bags under ordinary room temperature and moisture conditions. After the uninoculated oats were threshed, part of the glumed variety was dusted with spores without *first removing* the glumes, and part was dusted with spores after the glumes had been removed. A similar lot of the glumeless oats was dusted also. These supplies of inoculated seed were to be used for testing the relative ability of dormant spores, as compared with mycelium, to carry the pathogene over the winter. They were kept under exactly the same conditions as were the blossom-inoculated oats.

In the following spring, prior to sowing, the blossom-inoculated glumed oats were divided into two lots, one of which was deglumed to remove all pathogene structures which might be on or in the glumes.

In the spring of 1925 the writer had on hand, then, the following variously treated lots of seed oats, with definitely known history in so far as inoculation was concerned:

1. Swedish Select (glumed) uninoculated.
2. Swedish Select inoculated at blossoming time, glumes not removed and therefore with mycelium developed in the floral parts (possibly some ungerminated spores present).

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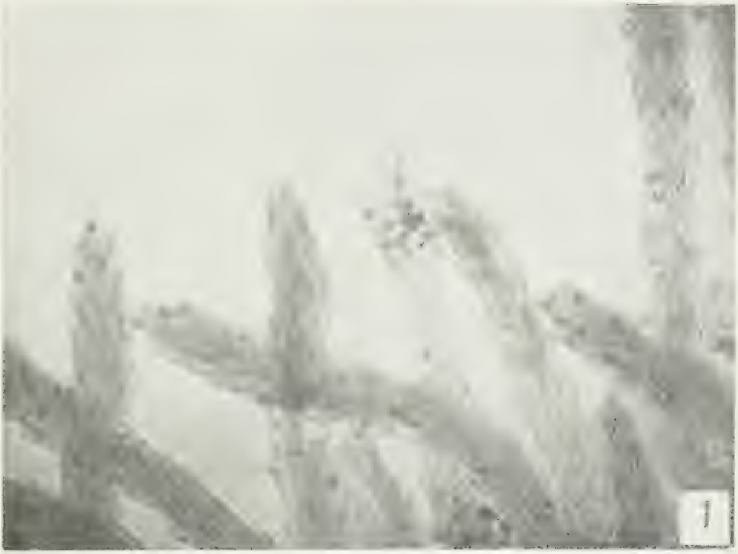
[2] Cotton blue (Blue cotton, G-4 b., Soluble blue) was used as follows: Solution A, consisting of 50 grams of phenol, 50 grams of lactic acid, 50 cubic centimeters of glycerin, 100 cubic centimeters of distilled water. Solution B, the same as A, with 1 gram of cotton blue. Material from water was placed in A for a few minutes, then transferred to B for a few minutes, and then returned to A for clearing.

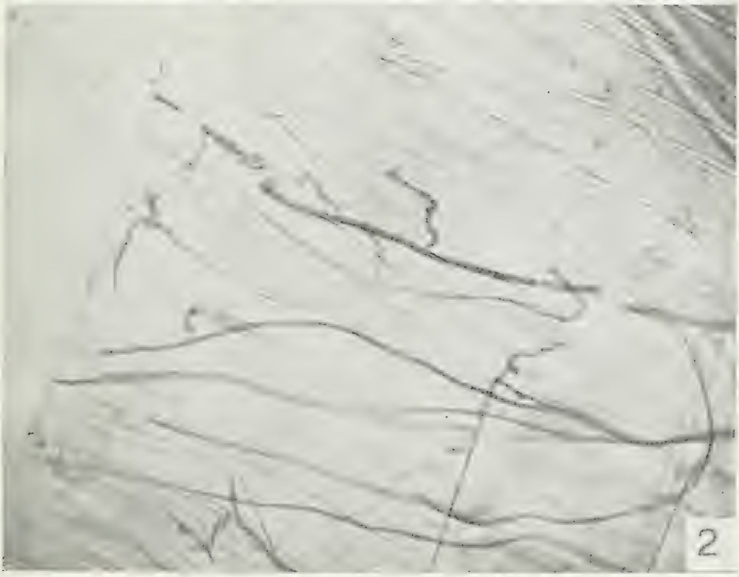
3. Swedish Select inoculated at blossoming time, glumes removed and therefore mycelium in the pericarp only (possibly some ungerminated spores present).
4. Swedish Select dusted with spores at harvest time, and therefore with ungerminated spores on the outside of the glumes.
5. Swedish Select dusted with spores after removal of glumes, and therefore with spores on the pericarp only.
6. Selection 202 (glumeless) uninoculated.
7. Selection 202 inoculated at blossoming time, and therefore with mycelium in the pericarp (possibly some ungerminated spores present).
8. Selection 202 dusted with spores after harvest, and therefore with ungerminated spores on the pericarp.

Ustilago levis.—In a like manner, lots of oats were obtained together with a supply of *U. levis* spores. Prior to inoculation the spores were examined as to their smooth-spored character. It may be well to note, however, that in obtaining spores for inoculating the first normal blossoms to appear, it was necessary to break open the smut sori. These covered sori do not open naturally until about the time when the last blossoms (largely from lateral shoots) are maturing. In the case of late-appearing smutted heads, the sori were not open at harvest time, and were broken open only by threshing. The possibility, then, of such spores entering the flowers lies in the fact that some of the first spores to mature are disseminated and lodge in the last of the flowers to open. Microscopic examinations following inoculation of flowers with *U. levis* spores disclosed germination and penetration of the floral organs identical with that described above for *U. avenae.* With glumeless oats the spores can, of course, come in contact with the caryopsis after pollination, or even during threshing. These points are discussed in more detail later.

The following seed supplies were obtained:

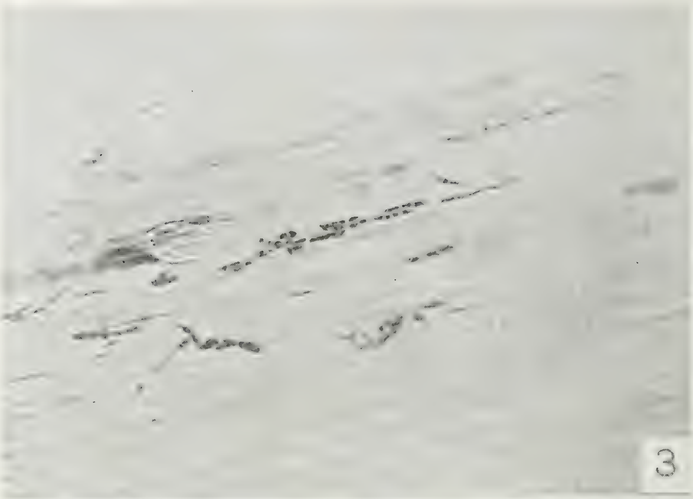
1. Swedish Select (glumed) uninoculated.
2. Swedish Select inoculated at blossoming time, glumes not removed and therefore with mycelium developed in the floral parts (possibly some ungerminated spores present).
3. Swedish Select inoculated at blossoming time, glumes removed and therefore mycelium in the pericarp only (possibly some ungerminated spores present).
4. Swedish Select dusted with spores at harvest time, and therefore with ungerminated spores on the outside of the glumes.
5. Swedish Select dusted with spores after removal of glumes, and therefore with spores on the pericarp only.
6. Selection 202 (glumeless) uninoculated.

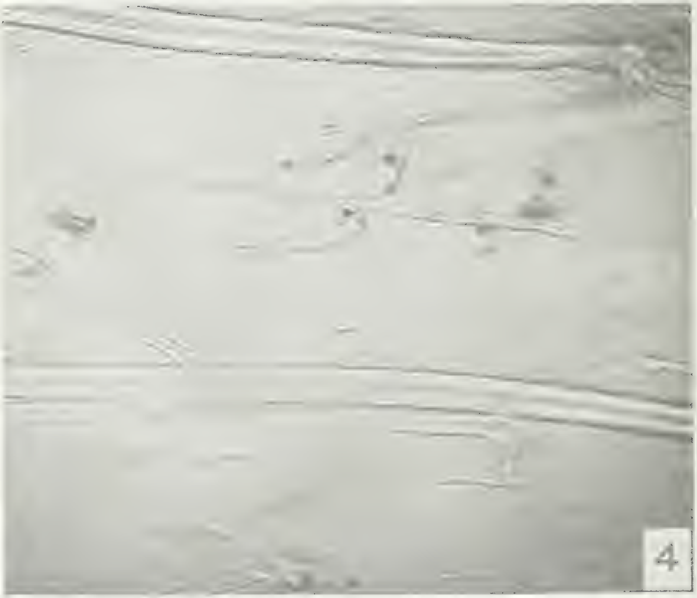




1, STIGMA BRANCHES OF THE OAT FLOWER (SWEDISH SELECT), SHOWING GERMINATION OF USTILAGO AVENAE SPORES AT POLLINATION TIME. ×225

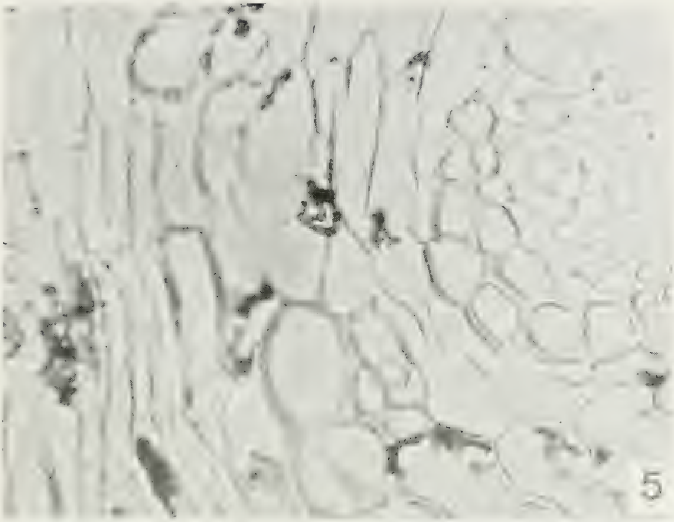
2, EPIDERMIS OF THE PERICARP OF A MATURE OAT (SWEDISH SELECT), SHOWING THE MYCELIUM OF USTILAGO AVENAE IN, ON, AND UNDER THE CELLS. THE EPIDERMIS WAS STRIPPED FROM THE CARYOPSIS AND THE PHOTOGRAPH TAKEN FROM THE INNER SIDE. ×225

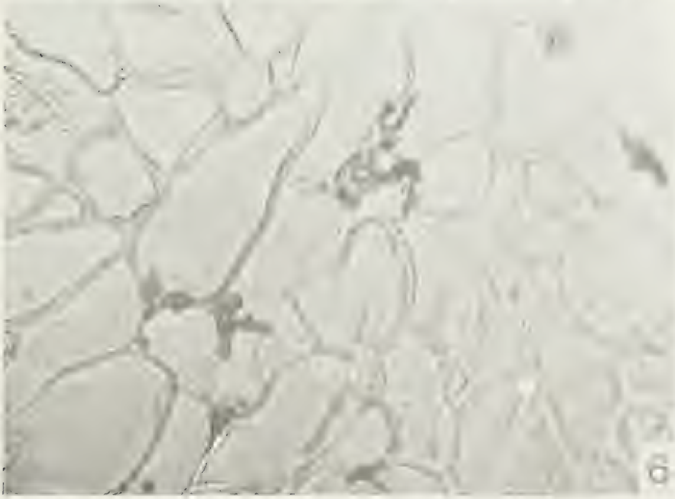




3, WING OF THE INNER GLUME OF A MATURE OAT (SWEDISH SELECT), SHOWING THE PRODUCTION OF "GEMMAE" FROM THE MYCELIUM OF USTILAGO AVENAE. ×265

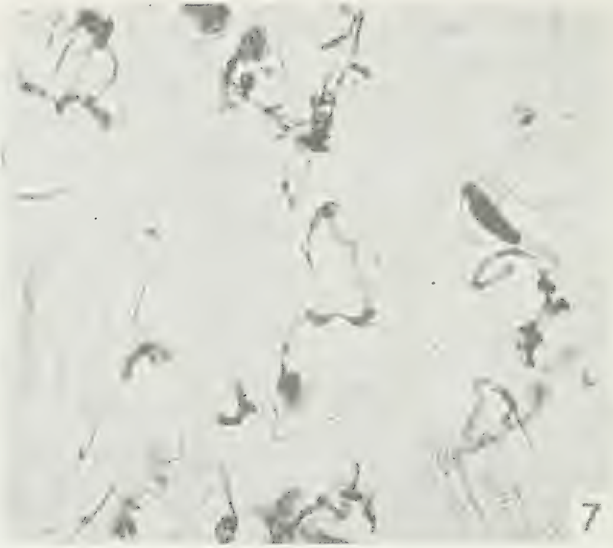
4, EPIDERMIS OF THE PERICARP OF A MATURE OAT (SWEDISH SELECT), SHOWING GERMINATION OF SPORES AND PENETRATION OF THE CELLS WHICH HAS TAKEN PLACE IN STORAGE FROM SPORES APPLIED AFTER HARVEST. ×225





CROSS SECTIONS THROUGH THE NODAL REGION OF THE CULM OF A SMUTTED OAT PLANT (SWEDISH SELECT) SHOWING THE MYCELIUM OF USTILAGO AVENAE (5) IN THE PARENCHYMA CELLS BETWEEN THE VASCULAR BUNDLES, AND (6) IN THE SCLERENCHYMA CELLS ADJACENT TO THE VASCULAR BUNDLES. ×265

The photographs were made from sections which were cut rather thick. This was done to emphasize the abundance of mycelium present. The lighter parts of the mycelium in the photographs are those parts which were not directly in the plane of focus. Their appearance is not due to degeneration



CROSS SECTION THROUGH THE NODAL REGION OF THE CULM OF A SMUTTED PLANT (SWEDISH SELECT), SHOWING THE MYCELIUM OF USTILAGO AVENAE IN THE PITH PARENCHYMA. × 265

The photograph was made from a section which was cut rather thick. This was done to emphasize the abundance of mycelium present. The lighter parts of the mycelium in the photograph are those parts which were not directly in the plane of focus. Their appearance is not due to degeneration

7. Selection 202 inoculated at blossoming time, and therefore with mycelium in the pericarp (possibly some ungerminated spores present).
8. Selection 202 dusted with spores after harvest, and therefore with ungerminated spores on the pericarp.

Experiments and results

Early in June, 1926, the variously treated supplies of seed, both those inoculated with *U. avenae* spores and those inoculated with *U. levis* spores, were sown in the greenhouse under conditions favorable for the growth and development of oats. Both pots and flats were used. All seed was planted approximately 1½ inches deep, and all plantings received frequent watering. Under these conditions and the high temperatures of the greenhouse in July, the plants began to head out in early August. The results are given in tables 2 and 3.

TABLE 2. SEED INOCULATED WITH USTILAGO AVENAE

Lot	Treatment	Number of seeds planted	Number of plants developed	Number of plants smutted	Per cent smutted
1	Swedish Select uninoculated	225	222	0	0
2	Swedish Select inoculated at blossoming time; glumes not removed, and therefore with mycelium in the floral parts (possibly some ungerminated spores present)	225	212	142	67.0
3	Swedish Select inoculated at blossoming time; glumes removed, and therefore mycelium in the pericarp only (possibly some ungerminated spores present)	225	208	128	61.5
4	Swedish Select dusted with spores at harvest time, and therefore with dormant spores on the outside of the glumes	225	223	4	1.8
5	Swedish Select dusted with spores at harvest time after removal of glumes, and therefore with spores on the pericarp only	226	221	26	11.8
6	Selection 202 uninoculated	100	97	0	0
7	Selection 202 inoculated at blossoming time, and therefore with mycelium in the pericarp (possibly some ungerminated spores present)	80	79	46	58.2
8	Selection 202 dusted with spores after harvest, and therefore with dormant spores on the pericarp	100	97	12	12.4

TABLE 3. SEED INOCULATED WITH USTILAGO LEVIS

Lot	Treatment	Number of seeds planted	Number of plants developed	Number of plants smutted	Per cent smutted
1	Swedish Select uninoculated	225	222	0	0
2	Swedish Select inoculated at blossoming time; glumes not removed, and therefore with mycelium in the floral parts (possibly some ungerminated spores present)	80	77	52	67.5
3	Swedish Select inoculated at blossoming time; glumes removed, and therefore mycelium in the pericarp only (possibly some ungerminated spores present)	80	74	49	66.2
4	Swedish Select dusted with spores at harvest time, and therefore with dormant spores on the outside of the glumes	100	98	3	3.1
5	Swedish Select dusted with spores at harvest time after removal of glumes, and therefore with spores on the pericarp only	100	93	27	29.0
6	Selection 202 uninoculated	100	97	0	0
7	Selection 202 inoculated at blossoming time, and therefore with mycelium in the pericarp (possibly some ungerminated spores present)	80	77	47	61.0
8	Selection 202 dusted with spores after harvest, and therefore with dormant spores on the pericarp	100	96	23	24.0

Deductions

Since the nature and position of the pathogene structures in each case is known, it is an easy matter to approximate the amounts of seedling invasion due to the various pathogene structures in the seed.

Ustilago avenae (table 2).—It will be observed from lot 3, in which seedling invasion was due to mycelium in or on the pericarp and possibly some spores adhering to it, that 61.5 per cent of smutted plants resulted, which is 91.8 per cent of the total smut obtained in lot 2. Since examinations of samples of this seed showed that very few of the spores had remained ungerminated, very little, indeed, of this 91.8 per cent could have resulted from overwintered spores. Furthermore, when deglumed seed was dusted with spores (lot 5), in which case they received exceedingly larger quantities of inoculum than could possibly have entered the flowers in the field, a comparative percentage of only 11.8 per cent of smutted plants resulted. Even this 11.8 per cent was not due entirely to spores which remained ungerminated, for it was observed here, as well as in all cases in which spores were applied after harvest, that not only had many of them germinated in storage but penetration and invasion of the pericarp also had taken place. Moreover, the 1.8 per cent of smutted plants resulting from spores on the outside of the glumes (lot 4) confirms the already well-established conclusion that such spores also play an insignificant rôle.

With lot 7 of the glumeless variety, in which the smutted plants arose from mycelium in or on the pericarp and possibly some spores adhering to it, a percentage of 58.2 occurred. Here again, as was learned from previous examination of seed, very few spores remained ungerminated, and hence very little of the 58.2 per cent could have come from dormant spores. Lot 8, which received greater numbers of spores than would have reached the kernels in the field, contained 12.4 per cent of smutted plants.

In both the glumed and the glumeless oats it is very evident, then, that the majority of the smutted plants were certainly due to the mycelium in or on the pericarp of the seed. Overwintered spores, as well as any pathogene structures in or on the glumes, play a very subordinate part in seedling invasion.

Ustilago levis (table 3).—In lot 3, 66.2 per cent of smutted plants resulted from mycelium in or on the pericarp and possibly some overwintered spores adhering to it. This 66.2 per cent represents 98 per cent of the total smut, or the 67.5 per cent, obtained from lot 2. Since very few of the spores were observed to have remained ungerminated after the inoculation of the flowers, most of the 98 per cent of infection was due to mycelium and not to overwintered spores. This is emphasized also by the fact that when deglumed seed was dusted with spores at harvest time (lot 5), in which case the seed received larger amounts of inoculum than did the blossom-inoculated seed, an infection of only 29 per cent resulted. The spores on the outside of the glumes (lot 4) produced only 3.1 per cent of smutted plants.

In lot 7 of the glumeless variety, 61 per cent of smutted plants were obtained from seed in which the mycelium in or on the pericarp, and possibly some adhering spores, were blamable. Since examination of this seed also showed that few spores do remain ungerminated after inoculation, the 61 per cent is certainly largely due to mycelium and not to spores. By way of contrast, spores dusted on the seed after harvest (lot 8) in amounts exceeding those applied at flowering time, resulted in only 24 per cent of smutted plants.

The procedure for *U. levis*, therefore, is practically the same as for *U. avenae*. The majority of the smutted plants are due to the invasion of the plants by mycelium already present in the pericarp of the seed from which they develop, and not from overwintered spores or pathogene structures in or on the glumes. It should be noted that in every case the resulting smut percentage for *U. levis* was somewhat larger than that for *U. avenae*.

Granting that some seedling invasion may result from spores which remain dormant, and also from mycelium in the glumes, it is still very evident that the majority of such invasions in both oat smuts are the result of mycelium developed in or on the pericarp from spores which lodge inside of the blossoms at pollination time. Careful examination of such seed revealed mycelium only in, on, or under the epidermis of the pericarp, and never in the embryo tissues. This absence of mycelium in the embryo is confirmed by the success obtained in controlling the disease by formaldehyde. If mycelium existed in the internal parts of the seed, the penetration of formaldehyde necessary to eliminate the pathogene would probably kill the seed also.

Since the formaldehyde treatment has been successful in the control of the loose smut of barley (Tisdale and Tapke, 1924: 264), it may be that the mycelium in the barley seed is also located largely in the pericarp. This has been suggested by these writers.

LIFE-HISTORY STUDIES

The evidence thus far has brought out the position, the nature, and the relative importance of the different pathogene structures which are present in or on the grain. Inoculation and incubation have been touched upon, but only in so far as was necessary for elucidation. For the sake of clarity, the writer believes that each phase of the life history of the two pathogenes—inoculation, incubation, and infection—should now be presented separately.

Inoculation studies

With Ustilago avenae

While natural inoculation was taking place, the writer spent considerable time not only in his own experimental plots but also in oat fields on the college farms, and made very careful observations. It was noticed that the spores of *U. avenae* are disseminated at a period beginning shortly before the healthy blossoms are open for pollination and continuing up until harvest. Most of the spores, especially those produced on the first, or main, culm of the smutted plant, are disseminated during the time when the first healthy blossoms are undergoing pollination, but the process is continued by later-appearing lateral shoots, and at harvest time there is still a considerable quantity of smut spores in the air.

The spores come in contact with all parts of the healthy plants, and a large majority, without doubt, either drop to the ground or are carried by the wind out of the oat field altogether. For the glumed-oat flower, there is a comparatively short period during which the spores may enter the blossom and come in contact with the floral organs. This period, the pollination period, varies approximately from twenty to sixty minutes, and as a general rule occurs during the late afternoon hours. Not all of the blossoms in a healthy panicle open at the same time, several days elapsing before all have undergone pollination. Spores, however, may lodge on the outside of the glumes at any time until harvest.

For glumeless varieties the inoculation period is very much longer. The spores may enter the blossoms at pollination time and continue to enter until harvest time. In addition, they may come in contact with the naked kernels at threshing time, when those spores which have not been disseminated in the field are shaken from the diseased heads that are harvested with the healthy oats.

With Ustilago levis

The inoculating process with *U. levis* is considerably different from that with *U. avenae*. The difference lies in the fact that with *U. levis* there is a much less developed correlation between the time of spore dissemination and the length of the pollination period. That this correlation may be more perfect under other climatic conditions than those that prevail in the area where these studies were carried on, is recognized. The sori containing the spores remain more or less intact until the majority of the blossoms have already undergone pollination. Even when the last blossoms are maturing, a very small number of spores are being disseminated.

With glumed oats, it will be readily seen that relatively few spores can reach the ovaries of the healthy flowers while they are still in the field. Some few additional spores, however, probably do get inside of the glumes when the oats are being threshed. By placing oats in a bag along with *U. levis* spores, very little shaking is necessary to bring about such a condition, especially in oats the kernels of which are not tightly enveloped by the glumes, or in those with the glume tips broken off. With glumeless varieties, the spores may reach the naked kernels in large quantities at threshing time. Since very few of the spores of *U. levis* are disseminated in the field, this inoculation at threshing time would exceed that from the same process with *U. avenae*, in which very few of the spores are not disseminated in the field.

It is evident, therefore, that these observations confirm those of previous investigators, but perhaps greater emphasis may now be laid upon the differences existing between the inoculation processes in the two species.

Incubation studies

With Ustilago avenae

As has been stated, those spores of *U. avenae* that lodge on the stigma and the ovary in the glumed oats germinate in a very short time. Those on the stigma, due to the excretions thereon, give rise to germ tubes sooner than do those on the ovary walls, but under the weather conditions prevailing at the time of the writer's observations the majority of the spores were fully germinated at the end of five days. No sporidia were formed, a result which may be explained by unfavorable temperature relations as brought out by Arland.

Penetration of the glumes and the epidermis of the pericarp was evident. By removing the pericarp epidermis and staining with cotton blue, the developing germ tubes could be found in all stages. Some were found outside, some were entering the cells, and some which had penetrated through the cells were established between the epidermis and the adjoining tissues. As is well known, in the development of the caryopsis certain tissue changes take place in the inner part of the pericarp when it is fusing with the integuments of the ovule. The writer is of the opinion that the mycelium passing through the epidermis of the pericarp is favored by these changes. Careful examination of the material (Plate I, 2) brings out this point. The mycelium, once it has passed through the epidermal cells, seems to grow and spread very rapidly, and it appears as if no interference takes place by intervening cell walls.

With glumeless oats, the incubation process for those spores which reach the ovaries at blossoming time is exactly the same as with glumed oats. In addition, as is brought out later in the discussion of epiphytology, those spores which reach the caryopsis in the field after pollination also germinate in a very short time and produce mycelium which invades the epidermis of the pericarp. This is true also of spores which do not reach the caryopsis until threshing time. The spores are capable of immediate germination, and favorable temperature and moisture relations for their germination usually prevail not only in the field but also in storage.

With Ustilago levis

It has been pointed out that relatively few spores of *U. levis* reach the ovary of glumed oats at blossoming time. However, those that do succeed in entering the latest blossoms to mature, behave as has been described for *U. avenae.*

In the glumeless varieties, the spores germinate, in all cases, shortly after they have reached the ovary or the maturing caryopsis. Without much doubt, this happens whenever they reach their goal while the oats are still in the field. The only exception to immediate germination would be cases in which exceedingly dry weather prevailed during the time of threshing, and when the oats were stored under exceedingly dry conditions. Examination of oats that had been dusted with spores shortly after threshing and stored in paper bags under ordinary moisture and temperature conditions prevailing in the office of the writer, showed a considerable amount of germination in the following spring (Plate II, 4). These points, however, are discussed more in detail later.

Infection studies

Since infection was found to be more or less alike in both loose and covered smut, the two processes are discussed together.

The time at which infection begins depends upon the time of penetration of the epidermal cells of the pericarp. This varies, as has been noted. However, the process is the same whether it begins shortly after pollination or as late as storage. As has been described under incubation, the mycelium passes into and under the epidermal cells. Just how much of this is to be included in infection it would be difficult to say, as no definite point marking the end of incubation and the beginning of infection can be established because no visible effect on the suscept tissues has been observed.

If infected seed is examined in the spring, it is seen that the mycelium appears to have developed to a considerable extent during the winter and can be found abundantly under or in the epidermis of all parts of the pericarp. It is evident, then, that the mycelium does not stay dormant throughout all of the winter, but under favorable conditions may be slowly growing and extending itself. The difference in the amount of mycelium at harvest time and in the spring confirms this conclusion.

In the spring, the mycelium thus developed in the pericarp brings about invasion of the young developing seedling. Just how this takes place will necessitate further study than has been made by the writer. The observations of Roesch (1926:387) must be discounted, since his methods are not in keeping with natural conditions. From the limited investigations that have been made by the writer, it would seem that the mycelium grows directly into the mesocotyl of the seedling. Since it is difficult to section material and retain the epidermis of the pericarp in place, a direct connection between a strand of mycelium in it and a strand in the seedling has not been observed. On the other hand, the very youngest seedlings in which mycelium could be detected were about seven days old and the invading mycelium was found only in the mesocotyl—that is, between the node from which the coleoptile develops and the node from which the first roots arise. In seedlings a little older, from twelve to fifteen days, mycelium was found in the coleoptile node and also extending a short distance down into the roots.

Should it be found that the mycelium grows out of the pericarp—that is, that it develops on the outside of the caryopsis prior to its entrance into the seedling—then the pathogene would have to be considered as having a two-cycled life history.

On examination of the culms of mature plants bearing smutted heads, mycelium is seen to be most abundant in the nodal regions and in the base of the leaf sheaths. It does not seem to favor any particular tissue, being present in the parenchyma of the pith (Plate IV, 7) as well as in that surrounding the vascular strands (Plate III, 5) and in that of the leaf sheaths. In addition it is frequently present in the sclerenchymatous tissues (Plate III, 6). It is prevailingly intracellular, although often found between the cells. This is contrary to the statements of Butler (1918:180) and Roesch (1926:389), but confirms those of McAlpine (1910:9). No true haustoria could be detected. In the internodes the mycelium is rather sparse and the hyphae are long and unbranched. In the nodes it is abundant and the hyphae are short, convoluted, and much branced. There is no evidence of mycelial degeneration and disappearance in the older or lower parts of the culms, as stated by Butler (1918:180). In fact the mycelium seems to be most abundant in the lowermost nodal regions.

The mycelial invasion of the developing inflorescence and the formation of chlamydospores has not been studied by the writer.

EPIPHYTOLOGICAL STUDIES

The effect of temperature and moisture conditions was recognized very early as having considerable influence upon the infection of oats by these smut pathogenes. The difference in opinion as to whether low temperatures or high temperatures favor seedling invasion is explained by the recent work of Bartholomew and Jones (1923), from whose investigations it appears that both extremes of temperature are unfavorable to the disease, the high, however, more than the low, and that maximum percentages of smutted plants result at from 18° to 22° C. Furthermore, these investigators show that low soil moistures, within a certain range of temperatures, favor invasion, while high soil moistures accompanied by high temperatures result in complete elimination of the fungus. The work of Reed and Faris (1924) is also important, since it emphasizes the fact that soil moisture, soil temperature, and soil reaction, interact as a set of factors, any one of which may be limiting. Statements with regard to early or late planting mean nothing unless they are accompanied by temperature and moisture records.

The experiments of the above-mentioned investigators, however, were carried on with oats which were dusted with spores just prior to sowing. Since seedling inoculation by spores can now be considered as taking place only in exceptional cases in nature, new data should be sought to explain the effects of environmental factors on seedling invasion. The writer has conducted a few experiments, which, although not conclusive, add something to the general knowledge.

First of all, dehulled and hull-less seed which had been dusted with spores just after harvest and which were subsequently examined in the spring, showed that the majority of the spores had germinated (Plate II, 4). Furthermore, penetration of the pericarp by germ tubes was evident also. From this it may be concluded that there are temperature and moisture conditions under which the pathogene may develop, but which are not conducive to the germination of the oat. Confirmation for this was sought in the following way:

Spores of *Ustilago avenae* and *U. levis*, on slides, were placed along with oats in petri dishes lined with wet filter paper. Both glumed and glumeless varieties were used. One set of dishes was kept for ten days at a temperature of 6° C., another at 10°, and a third at 18°. At intervals during the ten days the oats and the spores were examined for germination. In the set kept at 6° the majority of the spores had germinated, but only a very few of the oat seed had sprouted, and in these few cases the sprouting consisted of mere emergence of the radicle. In the set kept at 10° the spores had likewise germinated, and germination of the oats seemed to be taking place but at an exceedingly slow rate. At 18° both the spores and the oats had germinated rapidly. It is evident, then, that smut spores may germinate at temperatures well below those favorable for oats. (The term *germination* is used by the writer to mean emergence of the germ tube or the oat sprout with continued growth.) This does not support Butler's view, namely, that the spores require a considerably higher temperature for germination than do the oats, if his interpretation of oat germination is the same as that of the writer.

Similar experiments were conducted in which moisture was made the limiting factor. Six petri dishes were prepared with dry filter-paper linings. Spores and oats were introduced as in the previous experiments. To the first dish no water was added; to the second, one drop; to the third, three drops; to the fourth, six drops; to the fifth, nine drops; and to the sixth, twelve drops. The dishes were kept at a temperature of 18° C. for ten days. At the end of this time they were examined, and it was found that the spores had germinated in all dishes in which three or more drops of water had been supplied. In the case of the oats, only a few of those supplied with as many as twelve drops of water showed any germination at all. It is evident from this that spores are capable of germinating under moisture conditions which are deficient for the sprouting of the oat.

These experiments were followed by similar ones in which the spores were dusted directly upon the deglumed and the glumeless oat kernels. With temperature as a limiting factor, it was found that at 6° C. not only spore germination but also penetration of the pericarp epidermis took place, while the oats failed to develop sprouts. With moisture as a limiting factor, spore germination and penetration took place when three or more drops of water were added to the petri dishes, but below twelve drops the oats did not show any sign of germination. The amount of pericarp penetration was much greater than with temperature as a limiting factor.

From these data, it seems to the writer that one may be justified in concluding that infection may take place in storage by spores which reach the oats at threshing time. The ordinary manner in which oats are usually stored would certainly supply the necessary conditions of moisture and temperature.

In those experiments whereby the writer determined the position and the nature of the pathogene structures which carry the pathogene over winter in the seed, some of the blossom-inoculated seed did not produce plants with smutted heads. Examination of the culms of some of these revealed the fact that mycelium was present in their lower parts. Seedling invasion, then, had taken place, but for certain reasons the pathogene had not reached the inflorescence of the plant. This mycelium was found in most cases to be confined to the first node, but in a few cases it had reached the second and the third node. Since these oats were grown under conditions most favorable for rapid germination and development, it would seem that invasion of seedlings should universally result from infected seed. The importance of this lies in the fact that smutted *panicles can no longer be considered as a criterion of infection,* especially if variety tests are made in the field where absolute control of the plants is impossible. There might easily exist non-inheritable differences in some individual seeds which would effect faster germination. It will be remembered that Arland and Roesch noted that the inner and smaller kernels of a spikelet produced more smutted plants than did the outer and larger ones. That resistance of certain oat varieties may be explained by physical rather than chemical differences is also suggested.

As a further bit of evidence to show that smutting of panicles depends upon growth relations of suscept and pathogene, the following data are presented: Oats were sown and were permitted to attain a height of approximately six inches. They were then carefully transplanted after smut spores had been applied in the region of the first node. By infrequent watering a very slow growth was maintained. The plants resulting were rather stunted, but smutted panicles were produced in six of the nine thus inoculated. Checks were normal. Although such conditions would probably never take place in nature—except when the pathogene had failed to reach the growing point of the suscept at germination time, but succeeded in catching up due to an ensuing period of slow growth on the part of the suscept—this experiment provides additional evidence that smutting of panicles is dependent upon the growth relations of the suscept and the pathogene. It also refutes the statement of Lang (1913: 179) and of Butler (1918: 180), that the mycelium is halted by the maturing of nodal tissues.

Inasmuch as successful inoculation has been obtained by dusting spores on deglumed and glumeless seed prior to sowing, it may be well for the writer to present an explanation of why this is possible when under natural conditions seedling invasion is due to mycelium existing in the pericarp of the oat from which the seedling develops.

As has been pointed out, most investigators who have worked on seedling inoculation with spores recognized the fact that success depends to a large extent upon keeping their spore-dusted seed or seedlings at a low temperature prior to planting. Under such conditions the germination of the oat is retarded, while the spores germinate and produce germ tubes (or sporidia which in turn produce germ tubes), and then invasion proceeds as it would have done if mycelium had already existed in the pericarp. The process, after germ tubes are produced, is practically the same as would have occurred under natural conditions, but, since it takes several days for this development, the process as a whole is very uncertain. If the spore-dusted seed is immediately sown in the field, then the chances of infection which would give smutted panicles are decidedly lessened and not at all in keeping with nature. This has probably been the procedure in the major part of the work that has been done on varietal susceptibility, and, as has been indicated, the use of smutted panicles as a criterion of infection must be considered as a grave source of error in such work.

Aside from growth relations of pathogene and suscept, emphasis should be placed on another factor which has perhaps been overlooked or at least underestimated. In the case of glumed oats and *U. avenae*, it has long been recognized that only those spores which enter between the glumes and the ovary play a significant rôle as inoculum. Very frequently, in damp, cool weather, oat blossoms fail to open for pollination. The writer considers this to be a factor of great importance in the explanation of seasonal differences in amounts of smut.

GENERAL DISCUSSION AND CONCLUSIONS

To a certain extent, conclusions have already been indicated from the various data presented, but there are certain outstanding generalizations which should be more or less emphasized.

In the light of the discovery of spore germination at pollination time by Zade and his students, and the investigations of the writer, it is evident that the current conception of the life histories of *Ustilago avenae* and *U. levis* will have to be altered in several important respects.

In the first place, it will be necessary to remove the oat-smut pathogenes from the commonly called "seedling infection" group and place them in the "flower infection" group. Since they differ from the pathogenes in the latter group, however, in that the embryo is not invaded, the mycelium being restricted to the pericarp, it would seem that some distinction should be made. If a continuation of such groupings is advisable, the writer suggests that the "flower infection" group could be divided into an "embryo infection" subgroup and a "pericarp infection" subgroup. The oat-smut pathogenes could then be placed in the latter subgroup.

In addition, it must be recognized that infection of the pericarp is not restricted to the blossoming period of the oat. With *U. avenae* and glumed varieties of oats, this is largely the case. With *U. avenae* and glumeless varieties, infection may take place in the field at any time after pollination and even during storage of the oat. Since most of the spores are disseminated in the field, most of this infection would probably take place prior to harvest. With *U. levis* and glumed oats, the pericarp infection must be confined largely to the blossoming period. With glumeless varieties, it doubtless takes place also after the grain has been threshed since most of the spores reach the oats at threshing time, and therefore it is highly probable that much of the infection ensues in storage. In general, the length of the period during which inoculation is possible is without doubt the most important factor concerned with the resultant amounts of smut. All these points are in keeping with the writer's observations on the relative amounts of loose and covered smut during the years 1924 and 1925. Loose smut seems to be far more prevalent than covered smut among the glumed varieties of oats, while glumeless varieties have presented greater percentages of both kinds of smut but with the covered smut predominating.

Since the inoculation of glumed varieties of oats must, for the most part, take place during the period of pollination, it would seem that a failure of the blossoms to open for pollination would be a most important controlling factor in the amount of smut in the succeeding crop. This must very often take place, because either cool or damp weather at blossoming time prevents opening of the flowers.

In regard to other phases of environmental influence, further emphasis may be placed on the fact that temperature or moisture cannot be considered alone in an attempt to explain varying amounts of smutted plants in the crop. As is necessary in many other physiological studies, all factors which may influence the process must be taken into consideration before the effect of any one may be designated as limiting.

Of special importance is the fact that smutted panicles cannot be considered as an infallible criterion for infection, unless tests are made under conditions where absolute control of environmental factors is maintained throughout the entire development of the suscept. This, it would seem, is impossible under field conditions.

Considerable doubt necessarily arises as to the value of certain existing data on varietal susceptibility of oats to smuts. All work along this line should be checked by inoculating oats in accordance with the manner in which it happens in nature. If the artificial way of dusting seed with spores is continued, modifications should be introduced. The glumes should always be removed prior to dusting, and the inoculated seed exposed to conditions which will bring about that which would exist in nature, namely, infection of the pericarp before the oat starts to germinate. Comparisons of percentages of infection of naked and glumed varieties are worthless if spores are applied to the latter without first removing the glumes. Since there certainly must be differences in varieties which would influence the possibility of spores entering their blossoms, as observed by Diehl and Roesch, methods of inoculation approximating that in nature might change considerably the present status of our knowledge of varietal susceptibility.

SUMMARY OF CORNELL INVESTIGATIONS

1. Spores which reached the stigma of the oat flower at pollination time germinate at once or within a few days. This happens in glumed and glumeless varieties of oats, with both *Ustilago avenae* and *U. levis* spores.
2. Spores lodging on the ovary walls also germinate in a very short time.
3. With glumed oats the inoculation period is rather short, being restricted to the time during which the glumes are open for pollination.

Since some glumes are broken off by threshing, inoculation may take place to a certain degree at that time. With glumeless oats the inoculation period extends from pollination time until and during threshing.

4. The success of inoculation is dependent to a large extent upon the time of spore dissemination and the length of the period during which the spores may reach the ovaries or the caryopses. *U. avenae* spores are disseminated over a period extending from shortly before pollination until harvest. They must reach the ovaries of glumed varieties of oats during the time when the flowers are open for pollination, but may lodge upon the ovaries or the caryopses of glumeless varieties at any time after the flowers have opened. *U. levis* spores are not disseminated to any great extent until the very last of the flowers are being pollinated. With glumed varieties the possibility of the spores entering open flowers is very much less than for *U. avenae* spores. With glumeless varieties the majority of *U. levis* spores reach the caryopses of the oats during threshing.

5. Mycelium resulting from the spores germinating at blossom time may penetrate the glumes, but this mycelium is insignificant or at least unnecessary as a source of inoculum for seedling invasion, being entirely wanting in the case of glumeless oats.

6. Mycelium resulting from spore germination during the blossoming period or the maturing period of the oat, or even when the oats are in storage, penetrates and invades the pericarp of the caryopses and becomes established in and under the epidermal cells.

7. *The mycelium in the pericarp is accountable for most of the seedling invasion.*

8. The success of invasion resulting in spore formation (smutted spikelets) is dependent upon certain combinations of environmental factors, rather than on any specific one. In general, slow germination of the oat and continued slow development of the plant favors the pathogenes. Rapid germination and rapid growth of the plant, although they do not prevent seedling invasion and infection of the lower part of the plant, will often result in normal production of flowers.

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BUILDING UP RESISTANCE TO DISEASES IN BEANS

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The common field bean (*Phaseolus vulgaris* L.) as grown in New York is subject to a number of diseases and insect pests (Burkholder and Hawley, 1923). Two of the common and destructive diseases occurring in the State are anthracnose (caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav.) and mosaic (cause not known). With the work of Barrus (1911) it became known that there are two separate and distinct races of the anthracnose fungus which morphologically are identical but which biologically are very different. These races were named *alpha* and *beta*. From the practical standpoint, therefore, two separate anthracnose diseases were shown to exist. After extended experimentation, Barrus reported (1918) on the behavior of a large number of varieties of beans when inoculated with these two races of Colletotrichum and the plants placed in optimum conditions for infection and the development of disease. Only two varieties of field beans were found to be very highly resistant or practically immune for both races of the fungus. These were Wells' Red Kidney and White Imperial.

Subsequently, Burkholder (1923) reported a third race of *Colletotrichum lindemuthianum* which he named *gamma* and which has the ability to infect, among other bean varieties, both Wells' Red Kidney and White Imperial. The existence of the *gamma* race was not known at the inception of the work to be reported below.

Likewise the work of Reddick and Stewart (1918) has shown for the mosaic disease that, of a large number of varieties tested, only one showed complete immunity to the disease. This was a selection which was submitted for test by the late Professor Spragg, of the Michigan Agricultural Experiment Station, and which he finally described (1921) under the name *Robust*. This variety, like its progenitor Navy Pea, is susceptible to *Colletotrichum lindemuthianum*, race *alpha*, is practically immune to *beta*, and, fortunately for the outcome of this work, was found by Burkholder (1923) to be immune to race *gamma*.

The work of Burkholder (1918) and of McRostie (1921) on bean breeding for disease resistance, which was in progress in 1917, was well known to the writer. The progress of their work led strongly to the belief that suitable hybridizations would lead to the development of beans which would combine the resistance to anthracnose of each parent and the immunity to mosaic of one.

On the day (October 24, 1918) that Dr. Barrus terminated his inoculation experiments with the variety White Imperial, from which he concluded that this variety is highly resistant for the *alpha* and *beta* races of *Colletotrichum lindemuthianum*, he communicated this conclusion orally to the writer. Several plants both of White Imperial and of Robust stood on the greenhouse bench in full bloom. Reciprocal crosses were made on that and subsequent days as flowers reached a suitable stage for artificial cross-pollination.

MATERIALS AND OBJECTIVE

There are many dry-shell beans of various sizes, colors, and shapes on the market. In addition there are perhaps more which are grown in a limited way for home consumption. There is no reason to believe that many of these are really worth perpetuating, at least so far as intrinsic worth is concerned. It seemed desirable, therefore, to concentrate all effort on the production of a white bean which would conform to certain of the types now in large commercial production, such as White Marrow, Navy Pea, and Burlingame (Medium).

White Imperial does not conform to any of these types. The variety requires a long growing period to mature fully. This restricts the area in New York in which the variety can be produced at all, and, perhaps equally important, it interferes with a well-established and very satisfactory system of rotation which is followed in most of the New York regions of greatest bean production and which involves the fitting of bean land for seeding to winter wheat. In addition, the shape of the dry seed does not conform to any of the well-known types. It is oblong. It is not plump enough for White Marrow, nor for Medium. It is much too long for Navy Pea, and it is too straight and too short for White Kidney, a type which is fairly well known in the trade. White Imperial also is of determinate growth. This is regarded by some growers as an undesirable character. They claim, for example, that in case of unfavorable weather at flowering time there is more chance for the plants of indeterminate growth to set a crop. The exact importance of this factor does not seem to have been an object of study, and it may be questioned whether it more than offsets the advantage of having the pods held clear of the ground at harvest time when the crop may be destroyed by rot and mold following wet weather. Aside from its very high resistance to anthracnose, the variety has, to commend it as a parent, its very large and vigorous growth.

Robust, on the other hand, has much in its favor. It is a high-yielding typical Navy Pea type. It is immune to mosaic and practically immune to race *beta* (and subsequently was found practically immune to race *gamma* and also possessing more resistance to bacterial blight than most of the varieties in cultivation). The only characters that could be called objectionable are that the variety is very susceptible to race *alpha* and that it requires a slightly longer growing season than Navy Pea. The particular stock used for hybridizing was obtained from Professor Spragg under the numbers 40511, 40515, 40516, 40520. As grown at Ithaca these various lots were progressively prolific and progressively late in the order indicated. Of these lots, No. 40516 was used oftenest and is the one most involved in the subsequent account.

In a very few cases Wells' Red Kidney was used instead of White Imperial. The variety had nothing to commend it except its very high resistance to both *alpha* and *beta*. Both color and shape of seed are characters that affect the progeny adversely in that they yield sorts which must be discarded because they are off type. Likewise, the extreme susceptibility of this variety to the bacterial blights makes it an objectionable parent. The variety has determinate habit of growth, like White Imperial, and is, in consequence, subject to the so-called objections mentioned for that variety.

METHODS OF WORK

Pollination

Cross-pollination is easily effected. The work was done in the greenhouse, where no unusual precautions had to be observed. If the flower is emasculated when it is too young, it usually falls off; and if the pollination is made after the flower is open, selfing usually has occurred and no hybrid results. In the varieties employed the optimum time for pollination seemed to be just as the standards of the corolla begin to show signs of separating.

The seeds of both parents would germinate immediately on maturity, but the seeds of the first-generation hybrids usually would not. Some such seeds that had lain in soil for several weeks were finally dug up and the seedcoat cut through with a knife and partially removed. The seeds were replanted at once and thereupon germinated promptly.

Inoculation

Resistance to a single strain of anthracnose has been shown to be due to a single factor difference and to be a dominant character. The first generations were not tested, therefore, for anthracnose resistance. Neither were they tested for mosaic, since, at that time, McRostie's work (1921) did not permit him to say for a certainty just how mosaic resistance is inherited.

The second-generation plants were inoculated several times with a heavy spore suspension of races *alpha* and *beta* of *Colletotrichum lindemuthianum*. These plants were twice inoculated for mosaic, first on one juvenile leaf and after one or two days on the other.

Segregation

In the case of anthracnose a fairly sharp segregation was soon evident. All affected plants were discarded without record. Unfortunately, certain plants which did not show more infection than was produced on White Imperial by race *alpha* were saved for further test. Eventually all such progenies were discarded.

The segregation for mosaic was not so easily effected. The principal difficulty in this connection lies in the fact that there is no positive method of securing 100 per cent infection even on a susceptible variety such as Navy Pea. Furthermore, the symptoms of mosaic do not come out sharply in the greenhouse during the short cloudy days of winter, such as usually prevail at Ithaca, or in the field in summer if the weather is very warm and dry. On this account one lot of plants reached the sixth generation before it was finally discarded as susceptible to mosaic. Then, too, some of the plants, following White Imperial, did not show clear-cut cases of mosaic, and it became necessary to make inoculations from such plants on a susceptible variety.

Field tests

As soon as the quantity of seed warranted it—and usually this was in the fifth generation—the plants were grown in the field where they were subjected to the usual vicissitudes. When the lots were small, susceptible varieties (Navy Pea and White Marrow) were grown in the same row and inoculated; but later the susceptible varieties were mixed and grown in alternate rows. The anthracnoses were introduced as soon as the plants were well out of the ground, and in this way a very severe field test was secured in practically every year. In the early stages it was possible to inoculate every plant for mosaic, but later only a relatively small number of plants could be inoculated. Unfortunately, mosaic has never been observed to spread naturally in the test plots. Many susceptible varieties have been grown in adjacent rows every year, and Navy Pea, which is extremely susceptible to mosaic, has been used each year for a check and inoculated both for mosaic and for anthracnose. Small lots of plants were grown in the greenhouse from winter to winter and tested for mosaic. The progeny of such plants was marked for special observation the following summer in order to locate any that did not show clear cases of mosaic in the greenhouse. This is a very desirable precaution to take, as it is an excellent check on winter tests, due to seed transmission of the disease.

Isolation of types

Sorting for growth types, seed types, earliness and lateness, and so forth, began in the third generation. The sorting was not done too vigorously. Some lots that gave little promise were carried in small quantity from year to year, and in a few instances yielded, on further selection, types which have considerable promise.

RESULTS

In the summer of 1922, 58 selections remained in the test. All of these families were in the sixth to the tenth generation, the exact generation depending upon the number of times greenhouse cultures were produced. None of the plants was susceptible to either race of the anthracnose fungus under the most severe conditions that could be imposed in field or in greenhouse. They had been subjected to possible infection, from the stage of tender dispirited seedlings as grown in the weak light of midwinter, through a great variety of telluric conditions in the field, and had shown no infection whatever. They were immune. Also, so far as could be determined, they were immune for mosaic. There were lots of early-maturing and of late-maturing plants, plants of determinate and of indeterminate growth, seed types of typical Navy Pea and White Imperial with many intergrading types, one of which approximated White Marrow in size and was of the exact shape of this popular variety.

RACE GAMMA

Some of the lots had reached the stage where they might be put in a test for production, when race *gamma* was cultured and identified by Burkholder (1923). This race was isolated from White Imperial, but when it was found that it did not infect Robust it appeared that some of the hybrids might prove resistant. This seemed the more likely in view of the results which Burkholder (1923 : 322) had obtained with a Robust-Wells' Red Kidney hybrid and which he subsequently published. On August 11, 1922, and on September 15, a sample of each lot of the hybrids was inoculated in a large moist chamber with race *gamma*, the culture being very kindly supplied by Dr. Burkholder. The following record for ten consecutively numbered lots of plants will show what was found throughout this and subsequent tests. In these fractions, the denominator represents the number of plants inoculated, and the numerator the number that became infected—1/9, 3/10, 8/8, 4/5, 2/5, 2/5, 1/4, 0/2, 2/5, 5/5, and so on. In this test Wells' Red Kidney and White Imperial were severely affected, whereas Robust and White Sickel were entirely free.

All plants that showed resistance to the new race were grown to maturity and the progeny was grown in the field in the summer of 1923. All three races of the fungus were introduced early and gave good infection on the susceptible varieties. The season was very satisfactory for elimination of mosaic, but anthracnose never became abundant. Greenhouse tests were continued immediately after the field harvest. No crop was grown in 1924. In 1925 the following field notation was made: "Very heavy infection eventually resulted and by the end of the season the test could be called the most severe to which the hybrids ever have been subjected." A similar notation was made for the season of 1926, with the addition that the cold and wet summer gave valuable information on the behavior of the late-maturing varieties and on the relation of determinate and indeterminate growth to staining, mold, rot, and premature growth of seed.

CONCLUSIONS

The evidence presented above has been summarized from a mass of notes and records covering seven years. There seems to be no occasion to publish the notes in detail, but they will be preserved and may be consulted by any one who cares to see them. When it is borne in mind that for all practical purposes four separate and distinct diseases are involved in the work, it appears that material progress in building up resistance to diseases in common field beans has been made.

The relative ease with which the work has been done indicates that this method of attacking the whole problem of disease control in plants gives promise of a condition of permanent control which deserves the widest attention. That other investigators are coming to a similar conclusion is obvious from a perusal of disease-control literature of the past decade, and more particularly of the past five years. Considering the enormously successful results obtained in the classical examples of permanent control of disease—the European control of Phylloxera of the grape by the use of resistant stocks, the control of wilt disease of cotton, cowpea, and other plants by hybridization with a resistant parent, and so forth—it is a matter of surprise that even more attention has not been given to this method.

It is not to be concluded at all that the hybrids developed in this work will go at once into commercial production and eventually replace the present sorts. Some of the problems remaining to be considered are discussed in succeeding pages. In the meantime, however, any worker who desires samples of these hybrids may have them in small quantity for any further work along this line that he cares to undertake.

DISCUSSION

So far as the introduction into commerce of any of these particular hybrids is concerned, there are certain very definite things that remain to be found out.

Practical problems

Yield. Chief of the practical problems involved is, of course, the production record. A new variety that does not yield somewhat more than the average per acre of the standard sorts would not be accepted by growers. The present average reflects the losses in years of disease. A new variety which cannot equal the yield of the standard sorts in years when there is no disease clearly has very little to commend it. About the only advantage in the use of the new variety would lie in a tendency to eliminate fluctuation in yield (with attendant fluctuation in price), but as compared with the general character of the growing season and its effect on yield the elimination of the factor of disease might not produce a significant effect. At least this would be likely to be true in the case of such diseases as anthracnose, where severe losses occur only at intervals. In the case of a disease such as mosaic, however, the losses may be so great that the crop must be abandoned altogether. This was true in 1916 to 1918, in those parts of New York where Navy Pea beans were grown. Mosaic became so severe that this variety could not be grown. The substitution of the mosaic-immune Robust for Navy Pea occurred in these areas just as rapidly as seed of the new sort could be produced. In this case, however, the substitute is better than the original in every respect.

Minor objections to type of growth and the like might be easily overcome if the financial return can be shown to be adequate. But a variety that does not fit in readily with an established cropping system would have little chance to succeed.

Trade demand. The new variety must conform to the so-called "demand of the trade." While it may be true that a Bostonian housewife requires the variety Yellow Eye for baking, it is equally true that the demand of the average housewife rarely goes beyond a white bean or a colored one. This statement is easily confirmed at any public market. The demand for particular kinds of beans comes clearly from those consumers who use very large quantities of beans for canning purposes and the like, and from the dealers who must handle still larger quantities. The kinds of beans that are now on the market seem to satisfy present demands, and in general only the hybrid which conforms to some one of these types is worth offering to the public.

The quantity of seed now on hand is sufficient to begin comparative yield tests. After one more year the stock should be large enough for field tests in the various bean sections. The field tests are necessary because varieties that require more than one hundred days from planting date to harvest cannot receive a fair test at Ithaca, although they might mature very well in one hundred days if grown in a region where the total amount of radiant energy was greater for the period or even if it were distributed differently; and there are places in the State where the growing season for beans is one hundred and twenty days or more. Likewise those sorts that mature at Ithaca in eighty-five to ninety days might mature differently elsewhere. Furthermore, varieties that do not yield well on the heavy clay soil at Ithaca might produce very well on a different type of soil. Burkholder found this to be true in the case of Perry Marrow (oral communication).

Biological problems

Several of the problems just referred to as practical are essentially biological in nature but have to do with the inherent qualities of the plants themselves. There are in addition some external biological conditions that may bear on the problem.

Bacterial blight. If the hybrids should prove particularly susceptible to bacterial blight, their usefulness in New York culture would be decidedly limited. It is to be noted that Wells' Red Kidney is especially susceptible to this disease (or group of diseases) and that the advantage of anthracnose resistance is practically offset by the disadvantages of blight susceptibility.

Bacterial blight has been found in certain of the hybrids and a record made of its occurrence and severity. No systematic inoculation of the plots was undertaken, but the use of Wells' Red Kidney in alternate rows has given abundant opportunity for natural spread of the disease and it is likely that the absence or the infrequent occurrence of the disease in some of the hybrids suggests that they possess a considerable resistance to the disease—certainly as much resistance as Robust, which has been classed by Burkholder (1924) as among the more resistant of a large number of varieties which he has tested.

Root-rot. The root-rot disease, caused by *Fusarium martii* f. sp. *phaseoli,* is a factor to be considered in producing field beans in practically all parts of New York. Burkholder (1919) has shown that, in infested soil, annual losses from this disease range from 5 to 50 per cent of the crop. He has recorded also (1919: 1030) the existence of a resistant variety of very undesirable type. Although second-generation hybrids with this type were in existence as early as 1919, it is significant that no root-rot-resistant plant has yet been offered to New York farmers. Burkholder remarks that a very complex segregation has been found to exist in the hybrids made, and this doubtless has complicated the problem especially as the resistant parent is undesirable in a number of characters. McRostie (1921) suggests tentatively a two-factor hypothesis to account for the inheritance of susceptibility to root-rot. Thus far nothing further on this question seems to have been offered.

The hybrids in the present work have been tested to a limited extent in the greenhouse for root-rot toleration. The data show that some of the families are very susceptible and that others are decidedly resistant. It was inadvisable to inoculate plants in the field when the number was rather small, but now that the original purpose of the work has been accomplished the tests for root-rot tolerance can be executed although to do so means the introduction of the organism into land where it does not now exist.

Rust. In some sections of the United States the bean rust, caused by *Uromyces appendiculatus*, has been a serious factor in production. This disease seems never to have been important in New York. Random cases have been detected, especially on snap varieties and on Navy Pea. In two different years a trace of rust has been found on a few plants in the hybrid plots. In each case the infection probably came from Kentucky Wonder Wax or Golden Cluster Wax, both of which varieties have usually grown in close proximity to the hybrids and on both of which rust has been observed late in the season.

Anthracnose. That other races of *Colletotrichum lindemuthianum* exist has been demonstrated already by Leach (1923) and by Muller (1926). Unfortunately the varieties of beans employed by Leach in his work are not varieties that have been used by other workers nor are they known in the trade, so that there is no way of knowing at present whether or not the eight races that he describes are distinct from the three already known. Muller, on the other hand, obtained American material for comparison with four races which he isolated in Holland, and shows that the Dutch forms are different from those in New York. He also inoculated the seven differential varieties which were supplied him by Leach, and finds that two of his races behave alike on all seven varieties and therefore are like Leach's race VIII. But, from his tests with other varieties, he knows that they are biologically different. It is to be noted that Barrus found and recorded some variations in his work, but concludes (1918 : 597) that "we have been concerned here with only two strains of the pathogene." Leach (page 10 of reference cited) "does not consider this justifiable, because, if we consider difference in parasitism a sufficient basis for distinguishing biologic forms, a difference cannot justly be disregarded even if manifested on one host only. Furthermore, these forms may act differently on many other bean varieties which were not inoculated." Muller, in turn, quotes Leach's objection and uses it as a criticism of Leach's work. Just what the true situation is remains to be determined. Barrus points out that there may be variation within the host variety itself, and that a certain amount of cross-pollination which occurs naturally also may account for some of the variations which occurred in his tests. Of Leach's work it is to be noted that his conclusions in some instances are based on very meager data. It appears from his table 2 that his biologic form VII is established on a single inoculation of from three to six plants of the test variety. It appears also, from his discussion on pages 5 and 6, that the organisms used varied as much as two years in the time at which they were first brought into artificial culture. While there is no definite information that these races lose virulence in culture, there are inferential data on this point in the experience of those at this institution who have worked with the organisms. Burkholder (1925) has shown a positive case of attenuation in *Fusarium martii* f. sp. *phaseoli*, and the phenomenon is well known in the case of many bacterial parasites of animals and man.

The race which has been isolated at Bonn and studied comparatively by Böning (1926) corresponds most nearly with the *beta* race. But if the Leach formula is applied, it would appear that the Bonn race is distinct. Böning says (page 8 of reference cited): "Von den 14 in Amerika widerstandsfähigen Sorten müssen 2 als sehr anfällig gegenüber dem hiesigen Pilzstamm gelten, 4 weitere werden, wenn auch nur in geringem Grade, befallen, während die übrigen 8 Sorten auch wohl unter hiesigen Verhältnissen widerstandsfähig sind."

Although it seems somewhat unlikely (owing to the number of cases), there is, of course, the possibility that the seed which Böning obtained for his tests is not of the same varieties as were used by Barrus or Burkholder. Such mistakes in naming varieties are not uncommon, and, as is well known, different varieties are sometimes offered for sale under the same name. Furthermore, the condition described by Salaman (1926), for potato, may well apply in the case of beans. He records[1] that a newly introduced variety occurs in England which is partly immune and partly susceptible to the wart disease. Such a condition actually existed in the hybrids reported here before they were sorted for susceptibility to the *gamma* race.

Obviously, one of the next things to do is to make tests with all of the known races of *Colletotrichum lindemuthianum*. Even then, however, one could never be sure that a new race might not appear at any moment from some variety of beans which might be brought into a given locality. Such an event might be expected in the case of garden varieties, where novelties are being introduced almost yearly by a large number of seedsmen. In the case of field beans, however, the tendency seems to be in the other direction. Fewer varieties of staples are desired, in order that standardization may be effected. It is very unlikely that a new variety of dry-shell bean will be introduced in the fields of New York until it is known to conform to some one of the standard varieties now in cultivation, and even then it is not too much to expect that such a variety would not be accepted by growers unless they were assured that it was as good as or better than the standard, not only in yield and conformity to type, but also in resistance to diseases.

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[1] "It has happened within the writer's knowledge, that a newly introduced variety [of potato] consisted in reality of the product of two seedlings so much alike that no differentiating external features which would serve to distinguish them could be found, and yet one seedling was immune and the other susceptible to Wart Disease."—Salaman (1926:16).

Further, it is always possible that these hybrids might prove to be resistant to the new race. Certain varieties of snap beans always have been grown in close proximity to the hybrids, and some of these always have been sorts that were bought of seedsmen. One year, indeed, the organism was taken from these varieties of unknown origin and was mixed with the three races to make a general inoculation. No attempt was made to determine which races may have occurred on these varieties, but it was evident from the behavior of the plants used for check that nothing which could be called certainly new had been introduced.

OTHER RESISTANT VARIETIES

Finally, it may be added that in case none of these hybrids proves to be a desirable commercial sort and it seems impossible to secure a desirable type by the use of these parents, there exist some other varieties which possess a high degree of resistance to the three races of *Colletotrichum lindemuthianum* and which are very tolerant to mosaic. A large number of varieties have been grown for several years in a parallel row with the hybrids, and usually have been inoculated in the field along with the hybrids.

Nova Scotia Marrow. One of these varieties is in commercial production at the present time under the name *Nova Scotia Marrow.* The plant is determinate in growth habit, whereas White Marrow, which is susceptible to races *beta* and *gamma*, is indeterminate. The dry seed of the two varieties is very nearly of the same size and shape, and is readily interchangeable. An objection to Nova Scotia Marrow is that the cotyledons are likely to split apart somewhat, as the seeds dry down in the pod. The operation of threshing separates some of these, and, as a result, the pick is relatively high.

Some other varieties which have reacted like the Nova Scotia Marrow, but which from color or shape of seed do not offer such obvious possibilities, are listed herewith. These varieties mostly are not known in the trade and cannot be certainly identified by the use of Jarvis' work (1908).

Yellow-Eye Navy. A variety was procured from the John Lewis Childs Company in 1917 as new under the name *Yellow-Eye Navy.* The seeds at present approximate Yellow Eye in size and marking, and seem to be larger than when originally procured.

Soldier bean. A variety known as *Soldier* was procured from an exhibit of the New Hampshire Horticultural Society, and was said (in 1916) to be grown locally. The dry seed has the shape and appearance of White Kidney—white, with markings about as extensive as in Yellow Eye but of vinaceous color. The plants are determinate and large.

Sulphur bean.—A variety known as *Sulphur* was procured at the same time and place as the Soldier bean. In seed characters it corresponds most nearly with Eureka, but is smaller and more nearly round. It differs from Eureka also in its resistance to race *gamma.*

Leopard. Another local variety from New Hampshire, known as *Leopard,* is also very highly resistant to anthracnose. This is not the stringless snap bean described by Jarvis under this name, but a dry-shell variety. The dry seeds of this variety are about the size of White Imperial, but are colored on the ventral side a deep bluish purple, and have spots of this same color all over the dorsal side which otherwise is white.

Boston Beauty. Another local variety from New Hampshire is Boston Beauty. The seeds are somewhat shorter than those of Leopard, but they are marked like them except that the color is vinaceous red.

Jacob's Cattle. Another local variety from New Hampshire, Jacob's Cattle, is very much like Boston Beauty but the seeds are larger and longer and the seed is more nearly covered with vinaceous red color.

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THREE RUST DISEASES OF THE APPLE

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THREE RUST DISEASES OF THE APPLE

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The severity of rust on apples in the Hudson River Valley during 1927 and 1928 has led to a reawakening of interest in this problem. Concern is felt more particularly for the reason that abundant infections on the fruit have added enormously to the usual losses caused by foliage infection. The attention of the writers was especially drawn to the problem in the summer of 1927 by the appearance of numerous fruit infections on the variety McIntosh, which formerly had been regarded as highly resistant. The need for additional study of cedar rust was emphasized by the fact that the symptoms on McIntosh and other varieties were so unusual as to place in question the identity of the disease on these varieties.

As these studies have progressed, it has become evident that not one, but three, rust diseases are destructive among the varieties of apples grown in the Hudson River Valley. Since these three diseases and no others are found extensively on both cedars and apples in this region, and since no other rust disease is known to be prevalent on the apple in eastern North America, we shall assume that the rust problem in apples in the Hudson River Valley is fully represented by them. Accordingly, in this paper the three diseases are considered more or less in parallel rather than consecutively, in order to facilitate comparison and diagnosis. To avoid confusion the three rust diseases are designated as apple rust caused by *Gymnosporangium juniperi-virginianae* Schw., hawthorn rust caused by *G. globosum* Farl., and quince rust caused by *G. germinale* (Schw.) Kern.

PLANTS AFFECTED

Weimer (1917) has studied these three diseases on cedars and certain rosaceous plants in New York, but apparently found only the apple rust on apples. He did not, however, make extensive observations in orchards. Stewart (1910), in earlier observations in eastern New York during the period from 1895 to 1910, records only the apple rust on cultivated apples.

Authors' acknowledgments. Several workers have been very helpful in supplying specimens and information and in some of the inoculation experiments. In addition to those named in the body of this paper, we mention especially Professor W. L. Doran and Messrs A. B. Buchholz, A. B. Burrell, E. J. Hambleton, S. C. Jones, A. S. Mills, and E. V. Shear.

While many species of plants[1] are affected by one or more or these diseases, the number of species which have any important relation to rust of apples in the Hudson Valley is apparently much smaller.

Cedars

All of the three fungi are generally distributed on the red cedar (*Juniperus virginiana* L.) in the telial stage, and two of them, *G. globosum* and *G. germinale,* are perennial on the cedar. *G. germinale* is more evenly and abundantly distributed through the cedar areas than the other two species, which tend to be localized near apple or hawthorn trees. *G. germinale* may occur also on the juniper (*Juniperus communis* L.), but the juniper is not commonly associated with fruit trees in eastern New York and we have not encountered it.

Apples and related plants

Our observation on the aecial stages of these fungi, most of them made in 1928, indicate that the following are the principal suscepts in the Hudson Valley: for *G. juniperi-virginianae,* apple; for *G. globosum,* hawthorn (many species) and apple; for *G. germinale,* quince, hawthorn, and apple. We have rarely found *G. globosum* on pears, and in no case causing severe disease on this plant.

PREVALENCE ON VARIETIES OF APPLES, AND LOSSES

Many lists of susceptible and resistant varieties of apples have been prepared, but a considerable variation exists among these. Whether this variation is due to differences in the observers, to differences in behavior of the disease or diseases in different localities, or to differences in the fungi which produce the diseases, cannot be stated with certainty. It is clear, however, that varieties in the Hudson Valley which have until recently been rated as highly resistant, are now showing approximately as great an incidence of rust as are other varieties which are generally regarded as highly susceptible. It is apparent also that we are dealing with three distinct diseases, two of which are probably rare in many localities in which observations have been made. Another cause of the apparent or actual divergence of our records from those of earlier workers is the fact that we have given comparatively greater attention to infection of fruit than to that of foliage.

In the herbaria of the Department of Plant Pathology at Cornell University are two specimens of quince rust on apple fruit and three specimens of hawthorn rust on apple foliage, all from New York State. The earliest date for the quince rust is 1903, and the earliest of the hawthorn-rust collections was made in 1908.

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[1] Kern (1912) has assembled comprehensive lists of the species of plants known to be affected by these diseases. A few additions have been made since the publication of those lists.

Only one published account has been found to indicate that hawthorn rust occurs commonly on cultivated apples. Thaxter (1891), working in Connecticut, states "that perhaps the most common orange rust of apples in this State is undoubtedly caused by this species [*G. globosum*]." Professor Thaxter recently stated (in a letter dated November 9, 1928) that *G. globosum* on apple seems to be commoner farther south in New England than it is in southern Maine, where it very seldom occurs on cultivated apples but is not uncommon on wild stock. Dr. F. D. Kern also states (in a letter dated October 10, 1928) that *G. globosum* is not uncommon on apple foliage in the New England States. He has obtained collections from Connecticut, Maine, Massachusetts, New York, and Vermont. The only printed account which we have found of quince rust as an important disease of the apple, is presented in two brief notes from Nova Scotia by Hockey (1926, 1927). Dr. W. G. Farlow reported, in conversation with Dr. F. D. Kern a number of years ago, that *G. germinale* was not uncommon on apple fruits in the vicinity of Boston (letter from Kern dated October 10, 1928). Unfortunately, no more detail seems to have been preserved. Mr. Hockey has kindly submitted the following notes in a letter of October 18, 1928:

> Previous to 1924, apple rust was reported from Nova Scotia as caused by *Gymnosporangium juniperi-virginianae* Schw. A very careful survey in subsequent seasons has established that this disease is caused by *G. germinale* (Schw.) Kern. The alternate host for this rust is *Juniperus communis* var. *canadensis* Loud., a very prevalent plant in waste pastures and rocky slopes in the Annapolis Valley.
>
> The Roestelia stage was identified on Amelanchier, Crataegus, *Cydonia* spp., and on Gravenstein apple. It has been observed fruiting on Bishop Pippins, but no specimens have been examined by the writer. The disease is commonly observed at the calyx end of the fruit, causing a dark green, seminecrotic area. Very few fruit infections produce aecia, and infections on apple foliage have not been positively identified. Rust has been observed on the following varieties of apples: Crimson Beauty, Yellow Transparent, Gravenstein, McIntosh, Fameuse, Ribston Pippin, Bishop Pippin, Wagener, Tompkins King, Stark, Baldwin, and Russet.
>
> Another species of rust found on the low bush juniper, locally, is *G. claveriaeforme* (Jacq.) D. C. Aecia of this species have been found on *Amelanchier* and *Crataegus* spp. but not on apple.
>
> The red cedar, *Juniperus virginiana* L., is to be found in very scattered areas in Nova Scotia, and no rust resembling a Gymnosporangium species has been reported on this host.

Specimens of Gravenstein apple fruits bearing pycnia of *G. germinale* have been received from Mr. Hockey.

In view of the lack of knowledge of the hawthorn and quince rusts on apples, records of fruit and foliage infection are given here in some detail. No records of actual percentage of infected fruit were made by the writers in 1927. However, it was estimated that up to 50 per cent of McIntosh fruit was infected in some orchards. A. S. Mills[2] reports up to 8 per cent fruit infection on the McIntosh variety in Greene County at harvest time. W. E. Blauvelt[3] records 10 and 15 per cent fruit infection in several McIntosh orchards in Orange County. It is shown later, in this paper, that much of the infected fruit of this variety drops before harvest time. The remainder of the records presented here were taken during the season of 1928. Most of our observations of both cedar and apples were made in Columbia, Dutchess, Greene, Orange, and Ulster Counties.

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[2] Typewritten report of special field assistant, Departments of Plant Pathology and Entomology, Cornell University.

In table 1 are given some counts of quince rust on apple fruits collected in fourteen orchards in four counties. It will be noted that most of the varieties included in the table are usually listed as resistant or immune to the apple rust. From the data it is evident that direct fruit infection alone is blamable for heavy losses.

TABLE 1. QUINCE RUST ON APPLE FRUIT

Variety	Number of counts*	Per cent of infected fruit	Notes
Fameuse	1	21	
Hubbardston	1	28	
McIntosh	15	18	1 to 59 per cent, pycnia only
Winesap	2	74	72 and 76 per cent
Yellow Transparent	1	84	Pycnia only
Delicious	3	60	Pycnia only

*Counts for the most part represent 50 or 100 fruits.

In table 2 are shown some records of fruit infection by the apple-rust fungus. Even here the injury to the crop through direct infection of

TABLE 2. APPLE RUST ON APPLE FRUIT

Variety	Number of counts	Per cent of infected fruit
Jonathan	2	25
Rome Beauty	4	30
Esopus Spitzenberg	1	95
Sutton Beauty	1	27
Twenty Ounce	1	18
Wealthy	6	25
Winter Banana	2	12

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[3] Typewritten report of special field assistant, Departments of Plant Pathology and Entomology, Cornell University.

the fruit is obviously considerable, a fact which, with a few exceptions (Giddings, 1918, and Gardner, 1925), has escaped emphasis in the literature of apple rust. The varieties Baldwin, Greening, Williams, and Wagener have been found free of fruit infection in orchards in which both apple and quince rusts were present, at least in small amounts.

With the varieties of apples that have been studied, the quince rust causes distinctly more injury to infected fruits than does the apple rust. With some varieties, a large proportion of the fruits affected by the quince rust may fall from the tree before harvest time, and often the remaining fruits are rendered worthless. On June 27, 1928, the fruit on a McIntosh tree showed 33 per cent infection, while 47 per cent of the dropped apples under the same tree were infected. Again, on September 11, 1928, the fruit on a Delicious tree and the drops from the same tree showed 51 and 97 per cent infection, respectively.

The data in table 3 illustrate in another way, in part, the loss due to fruit infection on several varieties. The second counts were made in each case on the same tree or trees as were the first counts. A wide variation in the dropping of fruit is seen, even among the varieties affected by the quince rust. This difference in behavior is apparently parallel with the type of injury rather than with the variety of apple or the species of fungus involved. The symptoms on McIntosh and Delicious fruits are distinctly necrotic in type, while those on the varieties other than McIntosh in table 3 are usually hyperplastic or hypoplastic.

TABLE 3. DROPPING OF RUST-INFECTED FRUIT SHOWN BY SUCCESSIVE COUNTS ON THE SAME TREES, 1928

Variety	Rust	First count		Second count	
		Date	Per cent of infected fruit	Date	Per cent of infected fruit
Fameuse	Quince	July 5	21	Sept. 10	20
Hubbardston	Quince	July 5	14	Sept. 10	28
McIntosh	Quince	July 5	20	Sept. 10	6
McIntosh	Quince	June 27	14	Sept. 11	4
McIntosh	Quince	June 27	33	Sept. 11	8
McIntosh	Quince	June 28	31	Sept. 11	16
McIntosh	Quince	June 21	53	Sept. 10	28
McIntosh	Quince	June 27	65	Sept. 11	26
Jonathan	Apple	July 5	24	Sept. 10	38
Sutton Beauty	Apple	June 27	20	Sept. 11	27
Winter Banana	Apple	June 27	16	Sept. 9	14

No quince rust has been found on the foliage of apple, and no hawthorn rust has been seen on apple fruit. This is rather to be expected in view of the behavior of these diseases on hawthorn and quince. The hawthorn rust has appeared, however, with astonishing frequency on the collections of apple foliage which we have made (table 4). These collections were made in six counties in eastern New York, and at one station each in Vermont and Massachusetts. Foliage of only one variety (Wealthy) has been received from Massachusetts. Six of seven specimens representing as many varieties from Orwell, Vermont, bore only hawthorn rust. In a few instances this disease may be classed as severe, but more often the spots are small and sparse, with few aecia. This may account for the fact that the disease has generally escaped attention. In addition to the records included in table 4, the hawthorn rust has been found on seedling apple trees in two localities in Tompkins County, central New York. In one of these cases the infection was severe and decidedly predominant over the apple rust. Two collections of seedling apple foliage from different localities in the Hudson Valley bore only apple rust.

TABLE 4. APPLE AND HAWTHORN RUSTS ON APPLE FOLIAGE

Variety	Number of collections	Apple rust	Hawthorn rust	Size of spots	Notes
Baldwin	1	......	+	Medium	Slight
Ben Davis	1	+	......	Large	
Esopus Spitzenberg	2	......	+	Small	
Fameuse	2	......	+	Small	
Hubbardston	1	+	+	....	Severe, mostly apple rust
Jonathan	1	+	+	....	Moderate apple, slight hawthorn
McIntosh	8	......	+	Small	Mostly slight-moderate to severe one case
Northern Spy	1	......	+	Small	Slight
Northwestern Greening	1	......	+	Medium	Slight
Opalescent	1	+	......	Large	Slight to moderate
Rhode Island Greening	2	......	+	Medium	Slight
Rome Beauty	3	+	+	Large	Trace hawthorn
Sutton Beauty	1	+	......	Large	Slight to moderate
Tolman Sweet	1	......	+	Medium	Moderate
Tompkins King	1	+	+	Small	Slight
Twenty Ounce	1	+	......	Large	Severe
Wealthy	6	+	......	Large	Slight to severe
Winesap	1	......	+	Medium	Moderate to severe
Winter Banana	2	+	......	Large	Slight to severe

An interesting observation was made on the natural infection of McIntosh and Wealthy foliage in an orchard near Hudson, New York. On adjacent trees, the number of spots per infected leaf, on June 27, 1928, was 3.7 on Wealthy and 8.7 on McIntosh (table 5). On the McIntosh foliage, however, many of the infections had produced only small, pale areas, with a minute necrotic spot in the center. There was practically no defoliation on this variety, and yet only an occasional spot could be clearly distinguished on September 11. None of the spots had developed sufficiently to produce aecia; indeed, very few spots had progressed sufficiently to produce pycnia. At that time the Wealthy foliage presented the symptoms of a rather severe infection. From a comparison of the symptoms found here with those produced by artificial inoculations (described later), it seems probable that both the apple and the hawthorn rust were involved on McIntosh foliage, with the apple rust predominating. Here is apparently a case of resistance or hypersensitiveness similar to that encountered among the cereal rusts (Stakman, 1915) and elsewhere.

TABLE 5. RUST SPOTS ON ADJACENT TREES, MCINTOSH AND WEALTHY, JUNE 27, 1928

Leaf no.		1*	2	3	4	5	6	7	8	9	10	11	12	13	14
Variety	Terminal														
Wealthy	1	0	0	0	0	0	4	3	2	6	4	4	2	....	....
Wealthy	2	0	0	0	0	0	1	3	5	8	8	6	5	0	....
Wealthy	3	0	0	0	1	0	1	5	8	4	1	2	1	....	....
Wealthy	4	0	0	0	0	5	3	10	4	7	1	6	2	0	1
Wealthy	5	0	0	0	0	0	1	1	0	1	8	7	5	3	1
McIntosh	1	0	0	0	0	0	0	11	26	20	6	1	....	....	....
McIntosh	2	0	0	0	0	0	2	15	10	11	6	4	0	7	....
McIntosh	3	0	0	0	0	0	4	10	4	11	1	11	....	....	....
McIntosh	4	0	0	0	0	3	10	20	16	8	2	1	0	....	....

*Youngest leaf.

SYMPTOMS AND DIAGNOSTIC SIGNS

Considerable attention has been given by earlier workers to the symptoms produced by all these diseases on cedars (Weimer, 1917), and by apple rust on apples. It is not possible to give a complete treatment of the symptoms of quince and hawthorn rusts on the apple until observations can be made over a wider range of varieties and conditions. We shall consider here more particularly the characters which will be of service in diagnosing the diseases.

On foliage

Only the apple and hawthorn rusts have been found on apple foliage. On the upper (ventral) leaf-surface the spots are strikingly similar in appearance. However, on the varieties and seedling trees that have been studied, the hawthorn rust produces, on the average, distinctly smaller lesions (Plate I, 1), and none has been seen which equals in size those produced by the apple rust on a variety such as Wealthy. It is interesting to note that Wealthy foliage is highly resistant to hawthorn rust in inoculation experiments, the lesions produced being at most about 1 millimeter in diameter. On the lower (dorsal) leaf-surface, several distinguishing characters may be found. With the hawthorn rust the aecia are relatively few in number and are closely aggregate in the center of the lesion, and the adjacent leaf tissue in the lesion is of a light orange color. The peridial tubes are persistent and long, sometimes reaching 3 to 5 millimeters in length (Plate I, 2). These dehisce irregularly, never becoming markedly revolute.

In contrast, the apple-rust spots bear numerous aecia scattered over the lesion, sometimes forming a ring near the margin of the lesion, and the peridia are strongly revolute, especially when moist (Plate I, 6).

The two diseases can be readily distinguished by examining the terminal cells of the peridia mounted in water (Plate I, 3, 4). The differences are by no means so striking if cells are taken from near the base of the peridial tube. The terminal cells of *G. juniperi-virginianae* are relatively long and narrow, separate readily in water, and are strongly curved. The comparable cells of *G. globosum* are irregularly lanceolate in face view, and adhere in a pseudoparenchymatous plate when mounted in water, curving slightly or not at all.

On fruit

The usual symptoms produced by apple rust on apple fruit are well known. Gardner (1925) has figured and described symptoms on Rome and Gideon fruits, however, which are strikingly different from those commonly encountered.

The quince rust on apple fruits presents a wide range of symptoms which on different varieties may be almost completely dissimilar, varying from hyperplastic to necrotic but in the main hypoplastic or necrotic (Plate II). There is less tendency here for the lesions to be restricted to the calyx end of the fruit, and often several separate infections are found on a single fruit (Plate II, 11). The surface of the lesion is usually of a dark green color, seldom if ever becoming orange on the varieties that we have studied.

The hyperplastic and hypoplastic lesions produced by the quince rust usually involve considerable areas of the fruit and may be accompanied in later stages by marked shriveling of the fruit (Plate II, 8).

The necrotic lesions involve less of the fruit surface, the fungus apparently being arrested at a comparatively early stage in the development of the fruit (Plate II, 9). Thus an older lesion sometimes resembles bitter pit in superficial appearance (Plate II, 7). Not infrequently the necrotic tissue cracks to a considerable depth, sometimes exposing the seed cavities. In the interior of the fruit the necrosis is more extensive and tends somewhat to follow the larger vascular elements (Plate II, 10). Aecia have not been found on the extreme necrotic type of lesion, and pycnia are not abundant. In the hyperplastic lesions, pycnia and aecia may be present in considerable abundance.

The most definite and striking diagnostic characters are found in the fruiting structures of the fungus. The pycnia, aecia, aeciospores, and peridial cells of *G. germinale* are distinctly larger than those of *G. juniperi-virginianae* or *G. globosum*. The aeciospores in mass are of a bright orange color, in contrast with the dull brown color of the spore mass in *G. juniperi-virginianae*. The peridium of *G. germinale* dehisces by irregular shredding and does not become revolute. The peridial cells are wet with difficulty and adhere in a pseudoparenchymatous plate when mounted in water. In face view these are distinctly verrucose (Plate I, 5), in contrast with those of *G. globosum* which in face view are comparatively smooth in the center and rugose at the margins (Plate I, 4 A).

ETIOLOGY

Kern (1912) has published detailed descriptions of the three fungi under consideration, in a monograph of the genus Gymnosporangium. Our findings agree in most of the essential details with those of Kern. It seems desirable, however, to present in this paper certain characters in somewhat greater detail.

In figure 1 are shown the approximate frequency distributions of the three species in regard to length of aeciospores, based on measurements of 200 or more spores in each species. The aeciospores of *G. globosum* and *G. juniperi-virginianae* are similar in color and shape, and cannot be distinguished by casual examination. However, if 50 or more spores are measured and the mean length is computed, the two species are readily separated, since the mean length of *G. globosum* is roughly $21.60 \pm 0.17\ \mu$ while that of *G. juniperi-virginianae* is $27.45 \pm 0.01\ \mu$. The aeciospores of *G. germinale* have a mean length of $31.00 \pm 0.22\ \mu$, and in addition are lighter in color and more smoothly rounded than the spores of the other two species.

The aeciospores of the three species are more variable in width. The mean width of *G. globosum* aeciospores is $18.32 \pm 0.41\ \mu$, while that of *G. juniperi-virginianae* aeciospores is $22.67 \pm 0.51\ \mu$ and that of *G. ger-*

PLATE I

AECIA, AECIOSPORES, AND PERIDIAL CELLS OF G. JUNIPERI-VIRGINIANAE, G. GLOBOSUM, AND G. GERMINALE

1, Apple rust (right) and hawthorn rust (left) on leaves of wild apple
2, Aecia of *G. globosum* on a leaf of the Tolman Sweet variety
3, Peridial cell and aeciospores of *G. juniperi-virginianae.* × 160
3 A, Germinating aeciospores of *G. juniperi-virginianae*
4, Peridial cells and aeciospores of *G. globosum.* × 160
4 A, Peridial cells and aeciospores of *G. globosum.* × 275
5, Peridial cells and aeciospores of *G. germinale.* × 275
6, Aecia of *G. juniperi-virginianae* on leaf of cultivated apple

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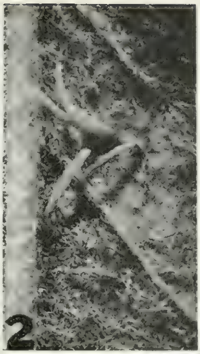
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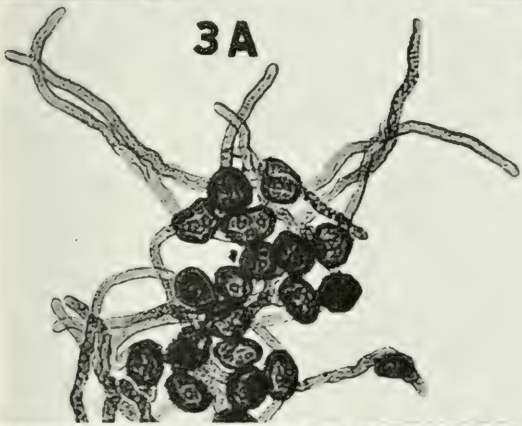
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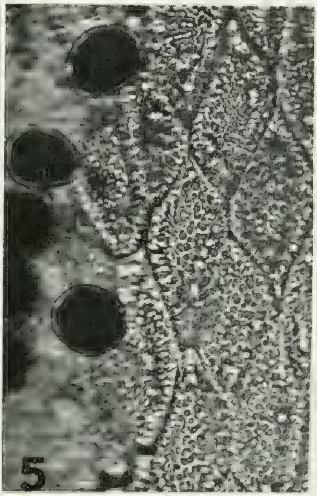
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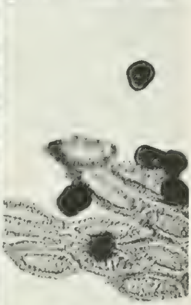
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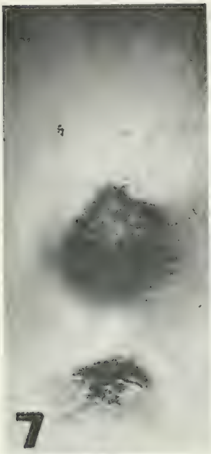

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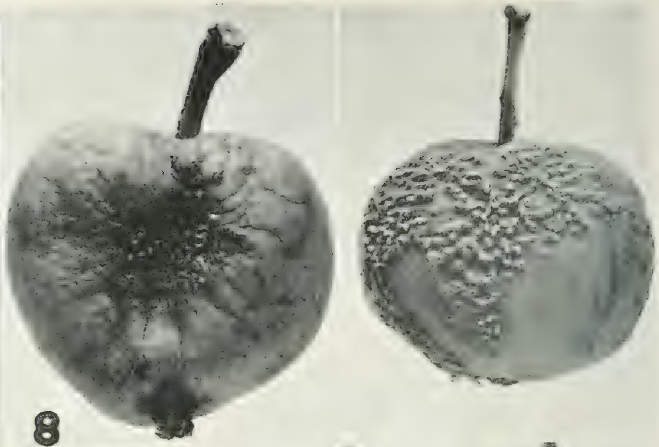

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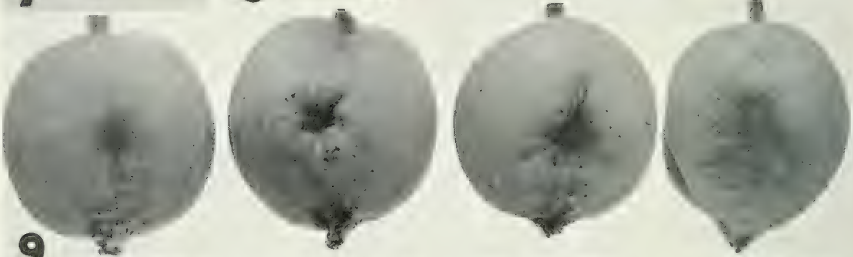

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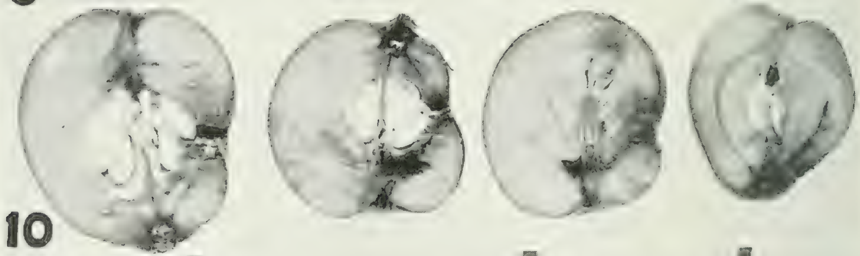

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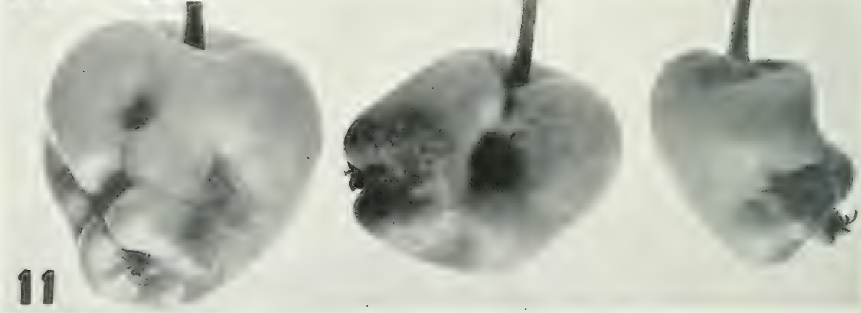

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PLATE II

QUINCE RUST ON APPLE FRUITS

7, Quince-rust spot on McIntosh apple fruit as the fruit approaches mature size

8, Quince rust on fruits of Winesap (left) and Fameuse (right), showing necrotic, hypoplastic, and hyperplastic symptoms

9, Quince rust on young McIntosh fruits, showing necrotic symptoms and pycnia

10, Cross section of young McIntosh fruits affected by quince rust, showing necrotic symptoms in interior of fruits

11, Quince rust on Delicious fruits

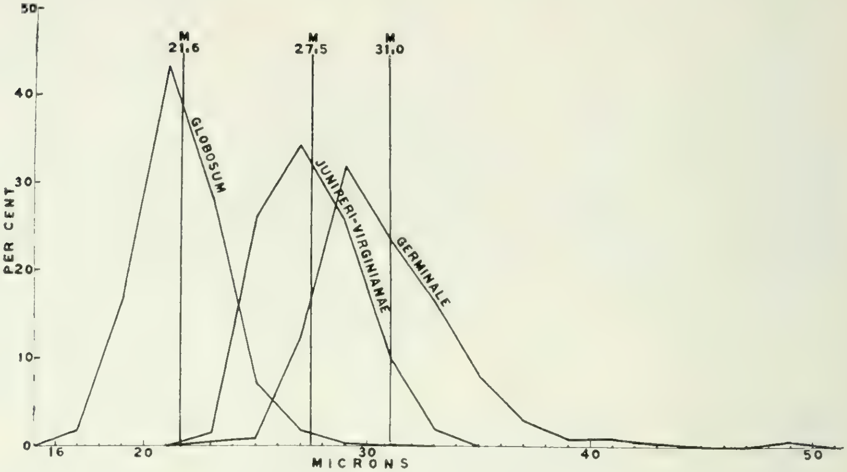

FIGURE 1. LENGTH OF AECIOSPORES

minale aeciospores is 26.88 ± 0.72 μ. Measurements of aeciospore width are less reliable as a means of identification.

An attempt was made to determine whether the spore dimensions of *G. globosum* are different on the comparatively resistant McIntosh apple from those on a susceptible species of Craetaegus. A slight decrease of both length and width on the resistant suscept was noted, but, with the number of spores measured, this decrease did not prove to be statistically significant.

The germination of aeciospores of these three species of fungi has been accomplished at best only with considerable difficulty (Reed and Crabill, 1915, and Weimer, 1917). Especially poor germination has been obtained by some workers toward the end of the growing season. A few casual attempts by the writers in late summer yielded negative results. However, it was noted that lesions of the apple and quince rusts on apple fruits kept in a moist chamber at 3° C. for several weeks,[4] seemed to enlarge somewhat and to continue the production of aeciospores. An occasional aeciospore was found to have germinated in situ. Since Fukushi (1925) has shown that exposure to low temperatures increases the percentage germination of aeciospores of *G. yamadae* Miyabe, it seemed probable that a similar relation might obtain for the species *G. juniperi-virginianae* and *G. germinale*. Accordingly, aeciospores of these fungi were taken from the 3° chamber and mounted in raised drops of distilled water at room temperature on December 6, 1928. A considerable number of the spores of both species germinated. More extensive tests were then made at various temperatures, and the results were indeed surprising (table 6, and Plate I, 3 A). We find no record, in the literature, of germination approaching that recorded here for the aeciospores of these species. The germ tubes were vigorous, and reached a length of 400 to 600 μ in twenty-four hours, even at 9° C.

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[4] The material of *G. germinale* was kept in the cold chamber for approximately twelve weeks, and that of *G. juniperi-virginianae* for about eight weeks.

TABLE 6. GERMINATION OF AECIOSPORES FOLLOWING EXPOSURE TO LOW TEMPERATURES (3° C.)

Date of test	Temperature (centigrade)	*G. juniperi-virginianae*		*G. germinale*	
		Number of spores examined	Per cent germination	Number of spores examined	Per cent germination
December 6, 1928....	21°	800	80.5	529	32.5
December 6, 1928....	15°	800	79.6	764	20.5
December 14, 1928...	12°	400	74.7	400	20.7
December 18, 1928...	9°	900	63.5	800	23.3
December 18, 1928...	6°	300	21.3	400	12.7

On December 14, fallen Crataegus fruits bearing *G. germinale* were collected at Ithaca, New York. The fruits were browned and more or less mummied in appearance, and the open aecia no longer contained spores. However, a few aecia were found which had never opened, and spores from these were mounted at 9°, 12°, and 15° C. for germination. Even here a germination of respectively 1, 2, and 3.5 per cent was obtained.

A single mount of aeciospores of *G. globosum* collected on December 12, 1928, on fallen Crataegus leaves, showed 54 per cent germination after twenty-six hours. The only appreciable germination reported for this species by Weimer (1917) was obtained from material exposed outdoors until November 8.

In view of these results and the successful inoculations made by Dodge (1918) and others, there seems no longer any reason to question that aeciospores of these fungi may produce infection during the season in which they are produced. It seems probable that the greater part of the natural infection on cedars will be found to take place in late autumn, rather than in midsummer or the following spring as has been suggested by various workers. Further studies are needed to provide direct evidence of the time and manner of infection of the cedar by each of these fungi.

It has been pointed out that the peridial cells of the three fungi differ in size as well as in shape and sculpturing (Plate I, 3, 4, and 5). The dimensions of these cells are more definitely recorded in figures 2 and 3.

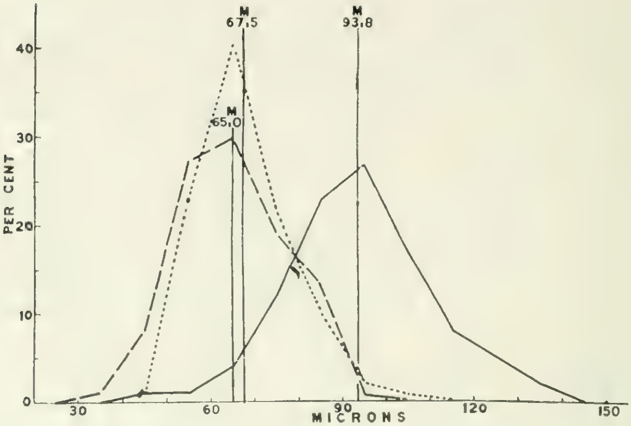

FIGURE 2. LENGTH OF PERIDIAL CELLS

——— *G. juniperi-virginianae*
— — *G. globosum*
.... *G. germinale*

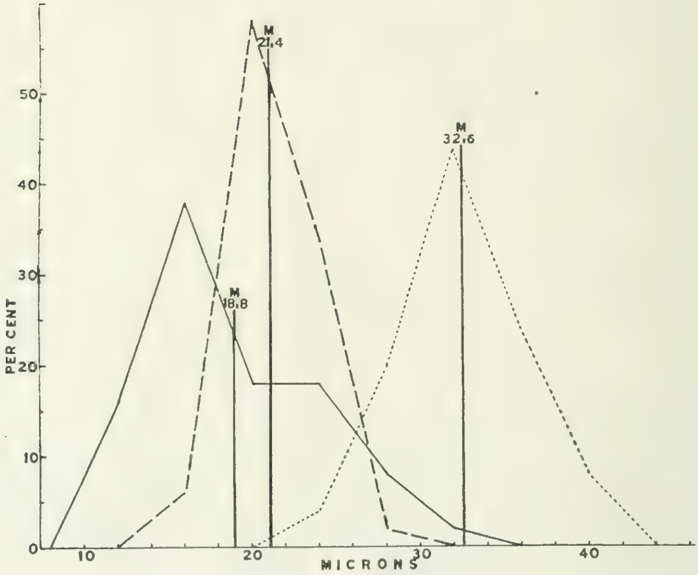

FIGURE 3. WIDTH OF PERIDIAL CELLS

——— *G. juniperi-virginianae*
— — *G. globosum*
.... *G. germinale*

These measurements were taken from cells rather toward the tip than toward the base of the peridium. There is a marked difference in size and shape between cells from the base and those from the tip of the same peridium, especially in *G. juniperi-virginianae*. This fact may account for the difference between these figures and those of Kern (1911).

The question of the existence of pathogenic strains among these fungi has received little attention, and no certain evidence bearing upon it has come out of this work. However, in view of the circumstantial evidence, including the variation in the results of inoculations with telial material from several sources, it seems desirable that this question be studied further.

Inoculation tests

It has been pointed out that this work was stimulated chiefly by the appearance on McIntosh apple fruits of a serious disease which for a time was not identified with certainty. When it seemed evident that the disease was a "cedar rust," several urgent questions arose. Chief among these was the question of determining which of the three rust diseases common on cedars in the Hudson Valley was blamable for the condition on McIntosh fruit. Accordingly, inoculations were made with the three fungi *G. juniperi-virginianae*, *G. globosum*, and *G. germinale*, on foliage of the McIntosh and Wealthy varieties in the greenhouse and on McIntosh fruit and foliage in the field.[5]

In seven fruit-inoculation trials involving several hundred fruits, no conclusively positive results were obtained. Most of these inoculations were made at least ten days after the blossoms had fallen, and were apparently later than the optimum period for natural infection.

On foliage, no infection was produced by *G. germinale* on McIntosh and Wealthy in the greenhouse (four trials) or on McIntosh in the field (four trials).

G. juniperi-virginianae was used to inoculate McIntosh foliage in the greenhouse and in the field in nine trials. Three inoculations were made on Wealthy in the greenhouse, for comparison. In two of the trials in the field, natural infection was not entirely excluded and hence no conclusive results were obtained from these. Of the remaining seven tests with McIntosh, six yielded infection varying from a few spots to as high as 74 spots on one leaf. In one test in the greenhouse, the infection on a McIntosh tree was sufficiently heavy to cause the dropping of several leaves in four weeks. These spots rarely reached a diameter of 1 millimeter, however, and for the most part merely developed into necrotic "flecks." Fukushi (1925) has shown that McIntosh foliage responds in a somewhat similar manner to infection by *G. yamadae*. No aecia of *G. juniperi-virginianae* have been found on McIntosh foliage from either artificial or natural infection. Inoculations on Wealthy foliage produced the usual symptoms associated with this fungus on a susceptible variety.

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[5] As in the case of other workers (Kern, 1911), the most satisfactory infection was obtained by suspending the inoculum above the parts to be inoculated in a humid but not stagnant atmosphere.

With *G. globosum*, ten inoculation tests were made on McIntosh foliage in the greenhouse and eight parallel tests were made on Wealthy. Six of the inoculations on McIntosh produced recognizable infection varying from a few spots to 46 spots on one leaf. On Wealthy, only one inoculation produced certain infection which was evidenced by a few minute orange spots (with pycnia) and many small necrotic spots. One other inoculation was followed by necrotic spots which resembled those of the preceding test but were not accompanied by pycnia or by orange color. Of three inoculations on McIntosh foliage in the field, two produced a small amount of infection but not equal to that produced by *G. juniperi-virginianae* in comparable tests.

In summary of the inoculation trials of McIntosh and Wealthy foliage, it is of interest to note that *G. germinale* did not infect either of these varieties, that *G. juniperi-virginianae* infected both varieties readily but the development of lesions in the McIntosh leaf was checked at an early stage, and that *G. globosum* infected both varieties but the Wealthy only with considerable difficulty.

During the latter part of the season of 1928, abundant rust material on a considerable number of varieties was available in the Hudson Valley. Thus a determination of the kind of rust involved was usually possible by the direct examination of aecia and pycnia of the fungi. Symptoms also were useful, within limits, as circumstantial evidence. As a result of all the evidence, it is concluded that the three diseases which are generally prevalent on the red cedar in the Hudson Valley are very common also on varieties of cultivated apples.

EPIPHYTOLOGY

The relations of rains and atmospheric humidity to infection, in the apple rust, are fairly well known. The relations in the hawthorn and quince rusts seem to be essentially similar. In the season of 1928, marked by a severe epiphytotic of all these diseases, the United States Weather Bureau Station at Hudson, New York, recorded, for the months of May and June, 1.69 and 7.89 inches of rain, respectively, as contrasted with 4.18 and 3.79 inches mean precipitation for that station. From May 18 to June 18 inclusive, 7.29 inches of rain was recorded, and from May 18 to June 6 there was never more than a one-day interval between rains. In 1927, when rust was rather severe, the rainfall was high in May (5.20 inches) and moderate in June (3.06 inches).

CONTROL

The control of apple rust by the eradication of the red cedar has been successfully practiced since the pioneer work of Jones (1893) and others. The similarities in the life histories of the three pathogenes, together with observations in orchards, suggest that the problem is essentially similar for the quince and hawthorn rusts. Considerable progress has already been made in cedar eradication by growers in the Hudson Valley, and a very considerable impetus will doubtless be given to this movement by the heavy losses of the past two seasons, not only on known susceptible varieties but on other varieties which have heretofore been considered practically free from rust. However, in a considerable number of cedar areas the cedar trees are in such great preponderance over the apple trees, or are held in such high esteem, that the method may still be considered impracticable. Compulsory state-controlled eradication of cedars has been employed in several cedar and apple areas in the Hudson Valley, but it has been attended by certain obvious difficulties.

Clearly, the most satisfactory solution of the problem lies in the eradication of the red cedar for a distance of at least one-half mile from apple trees, by individual growers or by neighbors in voluntary cooperation.

It has been stated that the apple and hawthorn rusts on cedars tend to be localized near wild apple and hawthorn. In some cases the removal of these plants from a cedar area would very markedly reduce the amount of inoculum produced by the cedars.

In areas where cedar eradication seems impracticable, the application of fungicides is the most promising alternate control measure. In two orchards, fruit of McIntosh were inclosed in paper bags on May 22, 1928, within a few days after the blossoming period (May 13 to 19). In each case the bagged fruits developed approximately the same amount of infection as did uncovered fruits on the same and adjacent trees. Thus, in 1928 at least most of the fruit infection on McIntosh was initiated before May 22 (about the time of the calyx application). This suggests that the susceptible period of fruit, for the quince rust, is passed in a relatively short time and during the time when applications of fungicide for scab control are most needed. In several orchards in which special attention was given to early spraying or dusting for scab control, on McIntosh particularly, the amount of fruit infection (rust) was low in comparison with other varieties and considering the exposure to sources of inoculum. In one instance a definite comparison was possible between adjacent rows of two blocks of McIntosh trees similar in size and in other respects except in the applications of fungicides. One block received four sprays (lime-sulfur) before blossoming, followed by the calyx and two later applications. The other block received only one application of fungicide (sulfur dust) during the entire season. The percentages of fruit infection on September 12, 1928, were 1 and 12, respectively. It is evident, then, that a considerable reduction in fruit infection may be expected when experiments are made with the timely application of fungicides. In this connection, more information is urgently needed concerning the time of maximum susceptibility of the fruit.

SUMMARY

It has been shown that the cultivated apple in the Hudson River Valley of New York is affected by three distinct rust diseases. These diseases are designated as apple rust caused by *Gymnosporangium juniperi-virginianae* Schw., hawthorn rust caused by *G. globosum* Farl., and quince rust caused by *G. germinale* (Schw.) Kern.

Both the quince and the apple rust cause direct and serious losses by infection of the fruit.

Hawthorn rust is occasionally destructive on apple foliage.

Varieties of apples show marked variation in their response to the diseases, both in type of symptoms and in severity of the disease.

Symptoms of the diseases and morphological character of the fungi are presented as aids to diagnosis. The terminal peridial cells of the fungi offer a means of rapid determination of the fungi.

Good germination of aeciospores was obtained after exposure to low temperatures.

Inoculation experiments were made with the three fungi on McIntosh and Wealthy foliage. No infection was obtained with *G. germinale*. *G. juniperi-virginianae* easily effected ingress into the McIntosh leaf as well as that of Wealthy, but was usually checked in the McIntosh before pycnia were formed. *G. globosum* infected McIntosh fairly readily, and Wealthy only with difficulty.

Eradication of red cedars seems to be the best method of control for all of these diseases.

The application of fungicides offers some promise in the control of fruit infection.

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THE RHIZOCTONIA DAMPING-OFF OF CONIFERS, AND ITS CONTROL BY CHEMICAL TREATMENT OF THE SOIL

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THE RHIZOCTONIA DAMPING-OFF OF CONIFERS, AND ITS CONTROL BY CHEMICAL TREATMENT OF THE SOIL[1]

James Stewart Wiant

In 1924 the writer began a study of the fungicidal properties of certain organic mercury compounds in comparison with various inorganic chemicals. Early experiments in greenhouses demonstrated that several of these materials were effective in controlling damping-off of coniferous seedlings. Field tests were then carried out in a small experimental nursery at Ithaca, New York, during the season of 1925. The results obtained suggested the advisability of continuing the investigations on a more extensive scale. An attempt was therefore made to determine the importance of damping-off in a number of the larger nurseries in the northeastern United States and the neighboring provinces of Canada. It was found that damping-off was a serious problem in seven of the eleven nurseries from which replies were received. In none of these seven nurseries, and in but one of the other four, were treatments with chemicals being systematically employed for controlling the disease. The need for further work in eastern nurseries was therefore apparent, and arrangements were made to establish a field laboratory at the nursery of the Keene Forestry Association, Keene, New Hampshire, where investigations were carried on during the seasons of 1926 and 1927.

Since Rhizoctonia was almost the only pathogene involved in these experiments, the larger part of the work was done on the particular damping-off disease caused by this fungus.

HISTORY

This review aims to note the more important work on the damping-off of conifers, and to call special attention to those papers having a direct relation to the present investigations. A more detailed review of the literature prior to 1921 may be found in the excellent paper of Hartley (1921).

The problem of damping-off early engaged the attention of German foresters and nurserymen, who first attempted the mass production of coniferous seedlings. Much of their work was observational in character.

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[1] Also presented to the Faculty of the Graduate School of Cornell University, June, 1928, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

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With the more recent development of reforestation projects and the consequent establishment of large coniferous nurseries in the United States, there has been a corresponding development of knowledge concerning this disease. Modern investigations have centered largely about the problems of etiology and control.

As pointed out by Hartley (1921:7), the only fungi definitely connected with the disease as it is discussed in the older European literature appear to be *Fusarium* spp. (*Fusoma* spp.) and *Phytophthora fagi* R. Hartig (*P. omnivora* De Bary in part). As far as the writer knows, *P. fagi* has not been reported from conifers in the United States.

Early reports of Fusarium damping-off in this country are those of Jones (1908), Gifford (1911), and Spaulding (1914). The last-named author finds forty-four different strains[2] capable of attacking pines. Hartley, Merrill, and Rhoads (1918) summarize the previous inoculation tests with Fusarium, and record the results of their own experiments with four common species. They agree with Spaulding that more than one species are involved and that *F. moniliforme* Sheldon (*Gibberella moniliformis* [Sheldon] Wineland) is one of the more virulent. More recently, Lindfors (1922) has isolated six species of Fusarium from pine in Sweden. His data indicate that these and two other species tested are pathogenic on conifers, but that four of the eight are decidedly more virulent than the others. Dounin and Goldmacher (1926) call attention to the prevalent opinion in Russia that but one species, *F. blasticola* Rostrup, is involved. From their tests they conclude that four additional species are pathogenic, but the data which they present are limited. It is of interest to note that these investigators claim to have isolated and studied a species of bacteria capable of producing damping-off in the absence of fungi. No details of their inoculation tests are given. Sibilia (1925), in Italy, has described several species of Fusarium from conifers, but evidently has not conducted inoculation tests.

Accounts of the relation of *Pythium debaryanum* Hesse to damping-off of conifers are given by Hartley (1910), Hofmann (1912), Spaulding (1914), and Hartley and Hahn (1919). In a later paper, Hartley (1921) reports extensive experiments which definitely prove the pathogenicity of this organism on pines. He demonstrates also that strains differ in their virulence, and shows that on the basis of morphology and pathogenicity the conifer strains as a group are not different from those attacking angiosperms.

Duggar and Stewart (1901) are evidently the first to have recorded the association of Rhizoctonia with the disease on conifers. Later reports are made by Hartley (1910) from Nebraska, and by Clinton (1913) from Connecticut. Hartley, Merrill, and Rhoads (1918), and Hartley (1921), conclusively demonstrate the pathogenicity of Rhizoctonia on conifers, and on the basis of inoculation tests point out marked differences in virulence between the strains used. Together with the work of Edson (1915) with sugar beets, and that of Edson and Shapovalov (1918) with potatoes, the results indicate that strains pathogenic on conifers are likewise capable of attacking angiosperms, and vice versa. Hartley (1921) concludes therefore that the strains from conifers are identical with the common *Rhizoctonia solani* Kühn, the perfect stage of which is *Corticium vagum* B. & C. (Burt, 1926).

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[2] The writer uses the term *strain* throughout this publication to indicate the fungus obtained in pure culture from a single isolation.

It is apparent from the literature (Duggar, 1915; Peltier, 1916; Rosenbaum and Shapovalov, 1917; Edson and Shapovalov, 1918; Matsumoto, 1921; Briton-Jones, 1924, 1925; Gratz, 1925; K. S. Thomas, 1925; Van der Meer, 1926) that marked differences exist between various strains of this common pathogene. The evidence presented by Rosenbaum and Shapovalov (1917), Matsumoto (1921), Gratz (1925), and Van der Meer (1926), suggests the existence of special forms within the species. Duggar (1915) and Matsumoto (1921) each report a single fungus which differs sufficiently from the others studied to possibly merit specific rank. Matz (1921), working with strains of Rhizoctonia found on subterranean plant parts, describes three new species which occur along with *R. solani*. From the work of Briton-Jones (1924), however, it would appear that the differences used by Matz in separating species are not greater than those between several cultures of the same strain of *R. solani* grown under different conditions. Most of the comparative studies on Rhizoctonia have been made with strains isolated from angiosperms.

Inoculation tests on coniferous seedlings with a number of miscellaneous fungi are reported in the papers of Hartley, Merrill, and Rhoads (1918) and Hartley (1921). More recently, Rathbun-Gravatt (1925) has brought together the results of direct stem-inoculation tests with more than one hundred different strains of fungi. Of these the author finds a few species of Fusarium and certain strains of *Corticium vagum*, *Pythium debaryanum*, *Pythium aphanidermatum* (Eds.) Fitz., and *Botrytis cinerea* Pers., capable of causing damping-off. The last three organisms, together with *Fusarium sporotrichioides* Sherb., are the most virulent. Further tests with this method are reported by Eliason (1928).

In summary, then, *Phytophthora fagi* and *Fusarium* spp. are reported as the chief causal organisms in Europe, and *Pythium debaryanum*, *Corticium vagum*, and *Fusarium* spp. in the United States. Hartley (1921) presents considerable evidence in support of his conclusion that these last-mentioned fungi are equally important as the chief causal organisms involved in the damping-off diseases of conifers in this country.

Many investigators have concerned themselves with attempts to control damping-off of conifers. While various methods have been employed, the most satisfactory results have been obtained through chemical treatment of the soil. A comprehensive discussion of this phase of control may be found in the paper by Hartley and Pierce (1917), who bring together not only the results of their own extensive experiments but also the published results of other investigators. Only the more important papers dealing with the use of sulfuric acid, formaldehyde, zinc chloride, copper sulfate, aluminum sulfate, and various mercury compounds, are discussed here.

Spaulding (1908, 1914), in experiments begun in 1905, devised the sulfuric-acid treatment, while Hartley (1912 b) made the first extensive tests with this material. Other publications by Hartley (1912 a, 1915), Hartley and Merrill (1914), and Hartley and Pierce (1917), have shown that of approximately fifty materials tested, sulfuric acid gave the most promising results. Not only was the acid effective in controlling the disease, but it reduced the number of weeds and caused increased growth of the seedlings. However, it was ineffective in alkaline soils, and in some nurseries caused chemical injury which necessitated frequent watering of the beds during the period of germination and emergence. In a more recent consideration of the original data, Hartley (1921) concludes that the necessity for this frequent watering can be eliminated if in each locality the minimum quantity of acid which will give reasonable control is employed. Later reports on the use of sulfuric acid are those of Hansen and others (1923), Toumey and Li (1924), Delevoy (1926), Wiant (1927), and May and Young (1927).

Early experiments with formaldehyde were made by Spaulding (1908, 1914), Jones (1908), and Gifford (1911). Hartley and his colleagues (Hartley, 1912 a; Hartley and Merrill, 1914; Hartley and Pierce, 1917) found formaldehyde effective, but less satisfactory than sulfuric acid. Similar reports are made by Hansen and others (1923) and by Toumey and Li (1924). The latter authors, as well as previous workers, noted considerable reduction in emergence on the formaldehyde-treated beds. May and Young (1927) reported that formaldehyde was superior to sulfuric acid. From their published data it appears that under the same conditions no treatment was made on more than a single plat.

Zinc chloride and copper sulfate were found effective by Hartley and his associates (Hartley, 1912 a; Hartley and Merrill, 1914; Hartley and Pierce, 1917), and were recommended by them for use on alkaline soils where sulfuric acid could not be employed. Hansen and others (1923) also report tests with these two materials, but unfortunately these investigators introduced a large number of varying factors into their experiments without providing for numerous replications.

Aluminum sulfate was successfully employed for the control of damping-off of conifers under the direction of Hartley (Anonymous, 1926). Similar results with this material were obtained by the writer (Wiant, 1927).

Hartley and Merrill (1914) report unfavorable results from a limited number of tests with mercuric chloride. In a later paper, Hartley (1915) points out that this material caused severe chemical injury, as was evidenced by the killing of dormant seed. The writer (Wiant, 1927), however, using much weaker concentrations than those employed by Hartley, obtained excellent results with mercuric chloride on coniferous seedbeds.

During the past several years, a number of commercial fungicidal preparations have appeared which contain as their toxic principle various organic mercury compounds, such as chlorophenol mercury, cyancresol mercury, and nitrophenol mercury. The first published account of the use of these preparations for the control of damping-off of conifers is that of the writer (Wiant, 1927), who points out their effectiveness when used both in solution and in the dry form. May and Young (1927), whose paper has already been referred to, used several of the organic mercury preparations in small-scale experiments.

The following papers deal with the use of a number of organic and inorganic mercury compounds for controlling Rhizoctonia causing damping-off of vegetable seedlings and brown patch of turf. Rosen (1924) states that chlorophenol mercury is effective in controlling Rhizoctonia damping-off of cotton. Gloyer and Glasgow (1924, a and b) found that mercuric chloride successfully controlled Rhizoctonia attacking cabbage. Clayton (1926), using chlorophenol mercury, cyancresol mercury, mercuric chloride, and nitrophenol mercury, failed to control damping-off in cruciferous seedbeds, but suggests as a possible explanation the fact that the first applications were made two weeks after emergence had occurred. H. E. Thomas (1927) found chlorophenol mercury and mercuric chloride effective for controlling Rhizoctonia damping-off in flats of cabbage and tomato. Small (1927) reports success with chlorophenol mercury in controlling foot-rot of tomato caused by Rhizoctonia. Coons and Stewart (1927) found that dusting sugar-beet seed-balls with chlorophenol mercury reduced the infection from soil-borne Rhizoctonia. A similar effect from liquid treatments with the same chemical on garden beets is reported by McWhorter (1927).

Both mercurous chloride and mercuric chloride were unsuccessfully employed in preliminary experiments by Piper and Oakley (1921) and by Oakley (1924) for controlling the brown patch of turf. Godfrey (1925) later demonstrated the effectiveness of chlorophenol mercury against that disease. Monteith (1925 a) reports similar results, but finds nitrophenol mercury ineffective. Monteith (1925 b, 1926), and Monteith and Harmon (1927), report extensive tests with a large number of organic and inorganic compounds of mercury, many of which, including mercuric chloride, were effective. They conclude, however, that mercurous chloride was the most satisfactory from the standpoints of control, toxicity, and cost.

PRESENT INVESTIGATIONS

DESCRIPTION OF THE NURSERIES

The Ithaca nursery consisted of twelve seedbeds located on the experimental farm of Cornell University. The beds were 4 by 12 feet in size, and were completely inclosed by a framework of wood which was covered with wire mesh. An additional half-shade frame was placed over the tops. The nursery was bordered by buildings and trees on three sides, but was open toward the south. The soil is a silty loam.

The Keene nursery, where most of the present investigations were conducted, is a large commercial nursery located near the city of Keene in southern New Hampshire. Situated along the southeastern terrace of the Ashuelot River Valley, this nursery is some thirty feet above the level of the valley bottom and is consequently well exposed to the prevailing west winds. Thus, excellent air drainage is provided over the greater part of the seedbed area. The soil is a medium sandy loam containing less than 3 per cent of organic matter and with a reaction of pH 4.8 to 5.2. This loam is underlain by nearly pure sand to a depth of eighteen inches, where it grades off into a coarse gravel. At about eight feet below the surface there is a layer of blue clay. Soil drainage is therefore rapid.

There are sown annually at this nursery approximately 50,000 square feet of seedbed, 70 per cent of which is devoted to norway spruce (*Picea Abies* [L.] Karst.), white pine (*Pinus strobus* L.), and red pine (*Pinus resinosa* Ait.). The remainder is used for colorado spruce (*Picea pungens* Engelm.), white spruce (*Picea glauca* Voss.), scotch pine (*Pinus sylvestris* L.), austrian pine (*Pinus nigra* Arnold var. *austriaca* Aschers. & Graebn.), and a number of other species, including balsam fir (*Abies balsamea* [L.] Mill.), concolor fir (*Abies concolor* Lindl. and Gord.), european larch (*Larix decidua* Mill.), engelmann spruce (*Picea Engelmanni* Engelm.), mugho pine (*Pinus mugo* Turra var. *mughus* Zenari), douglas spruce (*Pseudotsuga taxifolia* Britt.), american arborvitae (*Thuja occidentalis* L.), oriental arborvitae (*Thuja orientalis* L.), common hemlock (*Tsuga canadensis* [L.] Carr.), and carolina hemlock (*Tsuga caroliniana* Engelm.). From several points of view, norway spruce is the most important species grown.

The method of preparing the seedbeds is as follows. After the land has been plowed, harrowed, and fertilized, it is raked over by hand. Beds 4 feet wide and from 50 to 300 feet long are then laid out and separated from one another by 18-inch paths. The beds are raked, rolled, and marked out into drills running crosswise of the beds, 4 inches apart. Except for a few beds which are purposely arched in the middle, the level of the beds is little, if any, higher than that of the paths. Seeding is accomplished by walking down through the middle of the bed and sifting the seed into the drills from a long-necked bottle. A second rolling tends to close the drills and leave the seed covered with a thin layer of soil. Wherever this fails, a little soil from the paths is sprinkled on. The beds are then covered with a single thickness of burlap cloth, which is removed when emergence begins. The burlap is held in place and the beds are protected by wooden lath-covered frames placed flat upon the beds. These are provided with hinged legs, which can be used to elevate the frames some 8 or 10 inches above the surface of the beds after the burlap has been removed and the seedlings have begun to appear. The strips of lath covering the frames are spaced 1 inch apart, so that partial shade is provided during the two summers that the seedlings are left in the beds.

PREVALENCE OF THE DISEASE

The seedbeds at the Ithaca nursery were used for one season only. In order to insure damping-off, the beds were artificially infested with a culture of Rhizoctonia known to be pathogenic to red pine, the one species grown here. The disease was therefore extremely severe at this nursery. An almost complete loss of the seedlings resulted on the untreated plats.

From the time when the Keene nursery was established in 1906, until the spring of 1924, no serious damping-off was noted there by those in charge. In 1924 the disease appeared rather generally but caused heavy losses only in one corner of the nursery, where from 15 to 20 per cent of damping-off occurred in beds of white pine and norway spruce.

In 1925 the disease was very destructive in the red-pine and norway-spruce beds, resulting in losses of approximately 50 per cent and 35 per cent, respectively. These figures are rough estimates arrived at by the writer from observations on the final stand as noted during the summer of 1926. A section of the red-pine beds as they appeared when the above estimates were made is illustrated in Plate I, 1. The loss in the area photographed was practically 70 per cent. These particular beds were located near the area in which damping-off was severe in 1924.

The major part of the spring sowing of 1926 was made on a section of the nursery which had never before been used for seedbeds. Contrary to expectations, damping-off was far more severe than at any previous time in the history of the nursery. The seedlings on many beds were almost completely destroyed. Such an area of norway spruce is illustrated in Plate I, 2. The disease became evident with the initial appearance of seedlings of the earliest sowing, and continued to occur throughout the general period of emergence and for several weeks thereafter. It was manifested by an extreme reduction in emergence (particularly in norway spruce), and by the usual damping-off of seedlings after they had appeared above ground. In the case of white pine many seedlings were evidently destroyed by a rotting of the young roots when the stem tissues were already beyond the succulent stage.

The regular 1927 seeding was made for the most part during the preceding autumn, in a section of the nursery several hundred yards distant from the area where the disease had been prevalent in previous years. The incidence of disease was low in contrast with that of 1925 and 1926. The beds used for the experiments on control were, on the other hand, spring-sown and purposely located in infested areas. Severe damping-off occurred in most of the check plats. The relative importance of the two factors, location and time of seeding, is considered elsewhere in this work, but it is interesting to note here the pronounced difference in prevalence of the disease between the two areas just referred to. The losses from damping-off at the Keene nursery for the three years 1925 to 1927 inclusive, are summarized by species in table 1, those for the experimental beds where soil treatments were employed not being included.

TABLE 1. Losses from Damping-Off at the Keene Nursery from 1925 to 1927 Inclusive

Species sown	Year					
	1925		1926		1927	
	Area sown, expressed in number of 4-by-12-foot beds	Estimated percentage of damping-off after emergence	Area sown, expressed in number of 4-by-12-foot beds	Estimated percentage of damping-off after emergence	Area sown, expressed in number of 4-by-12-foot beds	Estimated percentage of damping-off after emergence
Austrian pine	10	....	13	None	28	5
Mugho pine	19	5–10	14	Severe*	5	Trace
Red pine	119	50	88	10–15	142	7
Scotch pine	25	5	42	None	32	5
White pine	294	3–5	445	30	192	5
Colorado spruce	6	80	38	45	45	10
Engelmann spruce	....	....	10	None	4	Trace
Norway spruce	225	35	311	50	456	7
White spruce	18	....	33	Severe*	26	Trace
Douglas spruce	168	....	....	....	....	....
Balsam fir	30	....	3	None	....	....
Concolor fir	....	....	12	None	....	....
European larch	....	....	42	Severe*	18	None
American arborvitae	20	....	31	None	13	None
Oriental arborvitae	17	....	17	Trace	13	None
Common hemlock Carolina hemlock	27	....	29	None	46	None

* The seed was of low viability, and so counts were not made.

The data for 1925 were estimated from the final stand one year after seeding, and are therefore only approximations. The figures for 1926 and 1927 were based partly upon estimates from careful observations during the season, and partly upon counts. They represent percentages of damping-off after emergence, on the basis of plants emerged, and are therefore low.

It is apparent that the disease has been most prevalent on norway spruce, white pine, and red pine — the three major species here grown — and on colorado spruce. Mugho pine, white spruce, and european larch suffered serious losses in 1926. The disease has been found also on scotch and austrian pine, as well as a trace on oriental arborvitae. Much of the variation between the same species in different years, and between different species in the same year, can be accounted for by the location of the seedbeds.

From these data one can obtain some idea of the losses that may result from this disease. There is in the first place a reduction in the quantity of salable stock. The tendency of most nurserymen in this situation is to sow large numbers of surplus seedbeds in order to maintain the production. This involves the added cost of seed, the price of which varies from $1 to $3 per pound for norway spruce and white pine, to $15 per pound for red pine; the increased cost of the seeding operations; and, most important of all, the greater expense of weeding and caring for the seedbeds during the two years between sowing and transplanting. There must then be considered the irregularity of production, which becomes particularly important in those cases in which long-term contracts are made for annually supplying large quantities of nursery stock. The combination of these factors serves to make such epiphytotics a problem of major importance.

ETIOLOGY

Isolation of organisms from diseased seedlings

Wherever possible, seedlings were collected for purposes of culturing the pathogenes soon after the seedlings exhibited symptoms of the disease. When the field work was first begun, the method of handling these was as follows. Short pieces of the stem were removed in such a manner as to include a part of the healthy tissue and an adjacent bit of the diseased tissue. These were immersed in a 1–1000 solution of mercuric chloride for one minute, rinsed in distilled water, and transferred to petri dishes of potato-dextrose agar. After several days, transfers were made from the margin of the advancing mycelium to other dishes of the same medium. The organisms were then transferred to agar slants. Since Alternaria, Fusarium, Pythium, Rhizoctonia, and other common fungi grew well on this agar, it was adopted as the standard medium throughout the course of these studies. Common saprophytes, such as species of Penicillium and Mucor, were discarded as soon as they were detected.

The method just described was somewhat modified in later work at the Keene nursery, the chief variation consisting in washing the sections of diseased stems with sterile water from a wash bottle and plating them

PLATE I

DAMPING-OFF LOSSES AT THE KEENE NURSERY

1, Section of 1925 seeding of red pine. All seedlings were destroyed in the immediate foreground and in scattered spots throughout the beds. Losses were estimated at 70 per cent. (Photographed at end of second growing season)

2, Section of 1926 seeding of norway spruce. A reduction in stand of approximately three-fourths of a million seedlings occurred on this area. (Photographed four months after seeding)

1

2

directly. The greater rapidity of this method permitted more extensive examinations than could otherwise have been made. The method was frequently checked by similarly examining sections of healthy seedlings. Such organisms as did occur on these plates were common saprophytes. Direct microscopic examination of diseased tissues made at frequent intervals also served as a check on the method of isolation here employed.

The results obtained from the Ithaca nursery are summarized in table 2 on the basis of fungi isolated from both untreated beds and those treated with chemicals for controlling the disease. The seedlings, all of which were red pine, were collected during the last three weeks of June, 1925.

TABLE 2. Summary of Isolations from Diseased Seedlings at the Ithaca Nursery

Treatments		Number of seedlings plated	Fungi isolated					
Materials used	Number of applications		Rhizoctonia	Pythium	Fusarium	Alternaria	Phoma	Unidentified
Untreated	.....	75	63	7	2	0	0	3
Sulfuric acid	1	56	31	8	3	8	3	3
Uspulun	2	31	0	20	1	5	3	2
Uspulun	3	33	0	12	1	5	5	10
Uspulun	6	29	1	10	2	7	4	5
Mercuric chloride	5	47	1	25	2	6	2	11
Bayer compound	5	48	8	23	2	4	1	10
Germisan	2	27	17	4	0	0	0	6
Total	.....	346	121	109	13	35	18	50

The data presented in table 2 indicate that Rhizoctonia and Pythium were the two fungi most frequently isolated at the Ithaca nursery, while Alternaria, Phoma, and Fusarium also were found associated with diseased seedlings. It is interesting to note that the seedlings from the untreated beds, and those treated with sulfuric acid and Germisan, respectively, yielded a much higher percentage of Rhizoctonia than of Pythium, while the reverse is true for those beds treated with other materials. Since the beds were artificially infested with Rhizoctonia, it would seem likely that under natural conditions Pythium would have been the most important organism involved in these beds.

During the season of 1926, extensive isolations were made at the Keene nursery from seedlings representing a large number of infection foci in beds of austrian, mugho, red, and white pine, colorado and norway spruce, and oriental arborvitae. Collections were made from June 1 to August 2, the majority, however, being made during the month of June. It is believed that the seedlings selected were typical in all respects of the large numbers which were destroyed during the epiphytotic of that season.

During the following season, collections were made from June 4 to June 23. As noted in table 1, the average loss from damping-off was low in the regular nursery seedbeds of that year, so that while many seedlings were collected from those beds the majority were taken from the experimental beds where the incidence of the disease was very high.

In addition to the seedlings collected at Keene during the season of 1927, specimens were at the same time received from a number of other nurseries as follows: white and red pine from Gerrish, New Hampshire; red pine from Milton, Pennsylvania; austrian and scotch pine from Amherst, Massachusetts; austrian pine, and colorado, engelmann, and norway spruce, from Framingham, Massachusetts; norway spruce from Essex Junction, Vermont; and japanese larch, norway spruce, and red, white, and scotch pine, from St. Williams, Ontario. These were collected from June 11 to June 21, and were for the most part in good condition when received. Isolations were made within a few days from the time when the seedlings were collected. While comparatively small numbers of the seedlings were examined in each case, the results give some indication of the organisms associated with the disease at these nurseries. A summary of these results, together with those from the Keene nursery in 1926 and 1927, is given in table 3.

TABLE 3. SUMMARY OF ISOLATIONS FROM THE KEENE AND OTHER NURSERIES

Location of nursery	Number of diseased seedlings	Number showing no growth	Number of fungi isolated						
			Rhizoctonia	Rhizoctonia and Fusarium	Rhizoctonia and Alternaria	Fusarium	Alternaria	Pythium	Penicilium or Mucor
Keene, N. H. (1926)	584	61	320	......	......	....	.....	.....	101
Keene, N. H. (1927)	734	30	594	......	......	6	.....	.....	79
Milton, Pa	45	.....	16	17	9	1	1	.....	1
Gerrish, N. H	105	.....	77	18	......	2	.....	.....	7
Amherst, Mass	104	2	45	28	......	14	.....	.....	15
Framingham, Mass	85	.....	3	......	......	51	.....	.....	......
Essex Junction, Vt	75	.....	1	......	......	37	.....	.....	......
St. Williams, Ont	93	.....	Few	......	......	Very many	.....	Many	......

The results from the Keene nursery are very striking, in that Rhizoctonia was isolated from the vast majority of more than a thousand seedlings. Although particular care was used in searching for Pythium, Fusarium, Alternaria, and other fungi which were found associated with the diseased seedlings at the Ithaca nursery, these organisms were rarely found at the Keene nursery in seedlings which were examined soon after they exhibited the first symptoms of the disease. Direct microscopic examination of such plants showed the tissues to be filled with the characteristic mycelium of Rhizoctonia. A number of the fungi noted above were obtained in a few cases from white-pine seedlings that had been killed several weeks previous to the time of collection. These miscellaneous organisms undoubtedly represented saprophytic development in seedlings already killed by the damping-off fungus.

The data in table 3 further indicate that Rhizoctonia was the chief organism associated with diseased seedlings obtained from the Milton, the Gerrish, and the Amherst nurseries, although Fusarium and Alternaria were both found to a lesser extent in one or more cases. Microscopic examination, however, showed that Rhizoctonia was more abundant than the other two organisms in the diseased tissues. The data for the other three nurseries are less detailed, but indicate that Fusarium was predominant in the diseased seedlings. Pythium was abundant in the seedlings from the St. Williams nursery. These specimens were in a rather advanced state of decay when they were received.

Inoculation and reisolation studies

When inoculation studies were first begun during the winter of 1925–26, the method employed was that of growing the seedlings in three-inch pots of unsterilized soil to which small amounts of inoculum had been added. Five pots were used for each organism, and three served as checks. The inoculum was prepared by growing the fungus for three or more weeks in flasks of steamed wheat kernels. Ordinary tap water was employed in sprinkling. In these experiments, considerable damping-off occurred in many of the check pots. Because of this, all subsequent tests were made in steam-sterilized soil and the number of check pots was increased from three to five. The pots were filled two-thirds full with a rather heavy loam, and steam-sterilized for two to three hours at 25 pounds pressure, following which 5 grams of inoculum was added to each pot. The inoculum was covered with a half-inch layer of a sterilized muck and sand mixture, and sufficient seed was sown to produce from twenty to forty seedlings per pot. The seed was covered with a thin layer of either sand or muck. During the winter of 1926–27, only distilled water was used in sprinkling, while ordinary tap water was employed during the following season.

For the first two winters the mean daily temperature of the greenhouse varied from 65° to 75° F., while for the third season automatic controls maintained a fairly uniform temperature of approximately 65° F.

Since the methods thus varied somewhat during the three winters, it should be pointed out that all of the Pythium strains and most of the other fungi from the Ithaca nursery were tested during 1925–26. The majority of the Rhizoctonia strains from the Keene nursery were tested in 1926–27, and all other fungi in 1927–28.

The results obtained from inoculation experiments with more than a hundred strains of fungi are brought together in table 4. While records

TABLE 4. RESULTS OF INOCULATION EXPERIMENTS WITH STRAINS OF FUNGI ISOLATED FROM DISEASED CONIFEROUS SEEDLINGS

Fungus tested	Conifer from which strain was isolated and on which it was tested*	Source†	Total number of strains tested	Number of strains showing differences in final stand as indicated below, between inoculated and check pots (differences expressed in percentage of seed sown)‡								
				80 to 90 per cent	70 to 80 per cent	60 to 70 per cent	50 to 60 per cent	40 to 50 per cent	30 to 40 per cent	20 to 30 per cent	10 to 20 per cent	Not significant
Rhizoctonia	R.P.	K	33	2	2	5	4	6	2	2	.....	10
Rhizoctonia	W.P.	K	20	.....	.....	.....	3	6	8	.....	.....	3
Rhizoctonia	M.P.	K	5	.....	.....	.....	.....	.....	.....	1	2	2
Rhizoctonia	N.S.	K	4	.....	.....	.....	.....	1	2	1	.....	.....
Rhizoctonia	A.P.	K	3	.....	.....	.....	1	1	1	.....	.....	.....
Rhizoctonia	C.S	K	4	.....	.....	.....	1	2	1	.....	.....	.....
Rhizoctonia	O.A.V.	K	2	.....	.....	.....	1	.....	1	.....	.....	.....
Rhizoctonia	R.P.	I	9	3	1	.....	1	1	.....	1	.....	2
Rhizoctonia	R.P.	M	3	.....	.....	.....	2	.....	.....	.....	.....	1
Rhizoctonia	R.P.	C	1	.....	.....	.....	.....	.....	.....	1	.....	.....
Rhizoctonia	A.P.	A	2	.....	1	1	.....	.....	.....	.....	.....	.....
Rhizoctonia	A.P.	F	1	.....	.....	1	.....	.....	.....	.....	.....	.....
Rhizoctonia (summary)	....	......	87	5	4	7	13	17	15	6	2	18
Pythium	R.P.	I	21	2	5	3	1	.....	1	5	.....	4
Fusarium	R.P.	I	5	.....	.....	.....	.....	.....	.....	.....	.....	5
Alternaria	R.P.	I	3	.....	.....	.....	.....	.....	.....	.....	.....	3
Sterile black mycelium	R.P.	I	3	.....	.....	.....	.....	.....	.....	.....	.....	3

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* Abbreviations used are as follows: A.P., austrian pine; C.S., colorado spruce; M.P., mugho pine; N.S., norway spruce; O.A.V., oriental arborvitae; R.P., red pine; W.P., white pine.

† Reference to nurseries are as follows: A, Amherst, Mass.; C, Concord, N. H.; F, Framingham, Mass.; I, Ithaca, N. Y.; K, Keene, N. H.; M, Milton, Pa.

‡ In the case of white pine, based on total viable seed.

were kept of the total emergence, damping-off, and final stand, the last only is included in the table. The difference between the average final stand in the five inoculated and the three or five check pots was thus calculated for each strain and determined in percentage of the total seed sown per pot. Since the viability of the white-pine seed had previously been determined as approximately 30 per cent, the results with this species are based upon the total viable seed.

Where the differences between check and inoculated pots were less than four times the probable error, the results were not considered significant. Such differences were for the most part very small. Those strains which produced a significant difference are grouped into eight classes according to the size of the difference. The figures in each column of table 4 therefore represent the number of strains that produced differences falling within the range of the class values indicated at the head of the column.

The data indicate that the vast majority of the strains of Pythium and Rhizoctonia were highly virulent under the conditions of these tests. This was manifested in the experiments both by reduced emergence and by increased damping-off in the inoculated pots. In the few tests with Alternaria, Fusarium, and an unidentified sterile fungus, these organisms were not found to be pathogenic. Other tests not included in the table indicated that three strains of Alternaria from red pine were non-pathogenic to colorado spruce, austrian pine, and scotch pine, while eight additional strains of Fusarium obtained from scotch and austrian pine were likewise not pathogenic. Of the eighty-seven strains of Rhizoctonia tested, forty-five gave differences of from 30 to 60 per cent while sixteen showed even greater differences in final stand between the inoculated and the check pots. It is interesting to note the somewhat wider range of virulence indicated for the strains on red pine than for those on other species. Similarly, in the tests with Pythium on red pine a wide range of virulence is to be noted.

Reisolations from several hundred diseased seedlings were made throughout the course of these experiments. In most cases the fungus used in the inoculation test was recovered from such seedlings.

The mass of evidence derived from isolation, inoculation, and reisolation studies demonstrates conclusively that Rhizoctonia is the principal pathogene involved in the nurseries where the experiments recorded in this paper were made, and throws additional light upon the question of the etiology of coniferous damping-off in this region.

Comparative study of some strains of Rhizoctonia

As pointed out earlier in this paper, most of the investigations dealing with comparative studies of Rhizoctonia involve strains isolated from angiosperms. Where conifer strains have been employed, attention has been chiefly devoted to inoculation tests performed for the most part on conifers. Comparatively little study has been made of their morphological characters. In view of the apparent differences between strains isolated from conifers during the course of these investigations, it has seemed desirable to make a comparative study of a number of these strains and a further comparison with several strains isolated from vegetables. The studies were divided into two parts: (1) cross-inoculation tests with thirteen strains on a variety of coniferous and vegetable seedlings; and (2) cultural studies with twenty-three strains on several artificial media. The conifer strains studied were selected as representative of the various types isolated. The several vegetable strains do not represent a wide selection from any particular group of suscepts. The twenty-three strains are listed below, together with the history of each. Those that are starred were used in both cross-inoculation tests and cultural studies, the others in cultural studies only.

Strain no.	Original suscept	Location of diseased plant	Year isolated
141	Austrian pine	Keene, N. H	1926
155	Austrian pine	Amherst, Mass	1927
*160	Austrian pine	Framingham, Mass	1927
*167	Colorado spruce	Keene, N. H	1927
175	Colorado spruce	Keene, N. H	1927
* 77	Norway spruce	Keene, N. H	1926
99	Oriental arborvitae	Keene, N. H	1926
* 13	Red pine	Ithaca, N. Y	1925
84	Red pine	Keene, N. H	1926
177	Red pine	Keene, N. H	1927
172	Red pine	Milton, Pa	1927
190	Red pine	Milton, Pa	1927
191	Red pine	Milton, Pa	1927
*192	Red pine	Milton, Pa	1927
*156	Scotch pine	Amherst, Mass	1927
158	White pine	Concord, N. H	1927
*187	White pine	St. Williams, Ont	1927
* 1	Cabbage	Eden, N. Y	1920
*184	Lobelia	Ithaca, N. Y	1927
*183	Pepper	Schenectady County, N. Y	1926
*193	Potato	Ithaca, N. Y	1927
*195	Sugar beet	Rocky Ford, Colo	1927
*196	Sugar beet	Rocky Ford, Colo	1927

The potato strain was isolated from sclerotia found on the surface of tubers; the lobelia strain from lesions on the stems and roots of that plant; the beet strains from lesions on the fleshy roots, those caused by strain 196 being of the dry-rot-canker type described by Richards (1921). All other strains were obtained from diseased seedlings.

With the exception of the following, all strains were isolated by the writer: the cabbage strain, which was a subculture of one isolated by

Gratz (1925); the lobelia strain, isolated by S. C. Teng; the beet strains, isolated by Dewey Stewart; and the pepper strain, isolated by Dr. H. E. Thomas.

Cross-inoculation tests

Thirteen strains of Rhizoctonia, including seven isolated from conifers and six from vegetables, were used in cross-inoculation studies. The species on which they were tested represent a selection from the more important species of plants commonly attacked in the seedling stage by Rhizoctonia. With two exceptions, white pine and lobelia, they include all of the suscepts from which the strains were originally isolated.

The methods employed were similar to those already described for the 1927–28 inoculation tests on conifers. The temperature of the greenhouse was maintained at approximately 65° F. throughout the course of the experiment. In testing each species of vegetable, three five-inch pots were inoculated with a given strain of the fungus, while an equal number were left as checks. The tests on conifers were conducted as previously described. The beet seed-balls were pasteurized at 60° C. for ten minutes on two successive days, in order to avoid infection from seed-borne fungi. It was not possible to carry out all of the tests simultaneously, but special efforts were made to maintain fairly uniform conditions throughout the course of the experiment.

The results of the cross-inoculation tests are found in tables 5 and 6, where the average difference between check and inoculated pots is given for each strain, this difference being expressed in percentage of seed sown. For sugar beets, however, it was found that in the check pots approximately one-third more seedlings appeared than the number of seed balls planted. Differences in final stand are therefore based on this expected stand, since it is impossible to know the exact number of seeds contained in a given number of seed balls. Because of the rather limited number of pots used in each test, differences that are less than four times the probable error are not considered significant.

As shown by the data in table 5, four of the seven strains from conifers (160, 77, 156, and 13) were highly virulent on conifers. In one case only (strain 77 on austrian pine) the difference is not significant - due, however, to contamination in several of the check pots. Strain 192 did not appear to be pathogenic to conifers. Strains 187 and 167, particularly the latter, were of relatively low virulence, as shown by the low average for all species tested and by the fact that in two and three cases, respectively, the differences are not significant.

Of the six vegetable strains tested on conifers, strains 183 and 196 were highly virulent, strains 1 and 184 were mildly virulent, strain 195 was of very low virulence, and strain 193 was non-pathogenic.

There is little evidence from the data in table 5 to indicate that a given strain is more virulent than others on the species from which it was origi-

TABLE 5. RESULTS OF INOCULATION TESTS ON SPECIES OF CONIFER SEEDLINGS WITH THIRTEEN STRAINS OF RHIZOCTONIA

Strain no.	Plant from which strain was isolated	Average difference between check and inoculated pots for each strain, expressed in percentage of seed sown					
		On austrian pine	On red pine	On colorado spruce	On norway spruce	On scotch pine	Average of the five
160	Austrian pine	79.2 ± 3.3	61.2 ± 3.9	65.6 ± 9.5	50.0 ± 2.4	36.0 ± 4.7	58.4
77	Norway spruce	54.4 ± 15.1	54.8 ± 7.9	62.4 ± 3.9	47.2 ± 2.6	43.2 ± 2.1	52.4
156	Scotch pine	77.6 ± 4.2	30.4 ± 3.5	56.4 ± 8.4	32.4 ± 5.1	53.2 ± 1.8	50.0
13	Red pine	68.8 ± 6.3	52.8 ± 4.7	40.0 ± 4.6	38.4 ± 1.5	36.8 ± 3.8	47.4
187	White pine	62.4 ± 11.7	–1.2 ± 6.1	29.2 ± 8.6	23.6 ± 3.8	42.0 ± 2.5	31.2
167	Colorado spruce	47.2 ± 4.2	19.2 ± 5.6	26.0 ± 7.2	28.0 ± 1.6	15.2 ± 5.5	27.1
192	Red pine	–1.6 ± 2.5	8.4 ± 4.3	–19.6 ± 11.2	4.0 ± 4.0	6.8 ± 5.1	–0.4
183	Pepper	81.6 ± 2.5	38.4 ± 3.8	54.0 ± 10.9	39.0 ± 2.7	32.0 ± 5.0	49.0
196	Sugar beet	60.8 ± 5.6	25.6 ± 5.6	72.4 ± 12.3	41.2 ± 3.4	39.2 ± 4.0	47.8
184	Lobelia	58.4 ± 2.8	20.0 ± 6.0	28.8 ± 7.4	26.4 ± 3.9	21.2 ± 4.4	31.0
1	Cabbage	41.6 ± 4.7	17.2 ± 3.0	23.6 ± 13.1	7.6 ± 5.1	....	22.5
195	Sugar beet	56.8 ± 6.3	20.0 ± 2.5	11.2 ± 14.1	–4.0 ± 6.5	22.0 ± 6.0	21.2
193	Potato	3.2 ± 2.0	–3.2 ± 7.5	4.4 ± 6.4	3.6 ± 3.7	6.0 ± 4.2	2.8

TABLE 6. RESULTS OF INOCULATION TESTS ON SPECIES OF VEGETABLE SEEDLINGS WITH THIRTEEN STRAINS OF RHIZOCTONIA

Strain no.	Plant from which strain was isolated	Average difference between check and inoculated pots for each strain, expressed in percentage of seed sown						
		On pea	On cabbage	On sugar beet	On eggplant	On pepper	On tomato	Average of the six
187	White pine	88.4 ± 4.1	80.0 ± 3.7	95.1 ± 6.9	49.3 ± 9.3	4.7 ± 3.6	73.3 ± 2.5	65.1
156	Scotch pine	73.4 ± 2.3	77.3 ± 1.8	....	16.7 ± 3.5	-26.0 ± 11.4	1.3 ± 2.9	28.5
77	Norway spruce	71.7 ± 9.9	26.0 ± 3.2	45.0 ± 9.3	28.0 ± 5.7	-14.0 ± 6.4	-0.7 ± 2.9	26.0
160	Austrian pine	68.4 ± 1.1	31.3 ± 6.0	9.9 ± 6.0	14.0 ± 5.4	-2.7 ± 1.8	10.0 ± 3.9	21.8
167	Colorado spruce	43.3 ± 9.2	18.7 ± 1.3	45.0 ± 10.4	2.7 ± 3.9	6.0 ± 6.8	8.0 ± 2.5	20.6
13	Red pine	41.7 ± 7.9	17.3 ± 2.2	6.9 ± 9.3	2.6 ± 5.2	5.3 ± 3.9	3.3 ± 4.0	12.9
192	Red pine	30.0 ± 6.4	32.0 ± 4.8	-23.1 ± 9.6	7.3 ± 6.1	1.3 ± 3.1	-1.3 ± 3.1	7.7
196	Sugar beet	98.4 ± 1.1	82.0 ± 3.4	81.0 ± 9.3	64.0 ± 3.1	32.7 ± 8.7	62.7 ± 2.2	70.1
183	Pepper	85.0 ± 3.2	69.3 ± 6.8	75.0 ± 7.0	67.3 ± 5.9	57.3 ± 6.5	45.3 ± 3.1	66.5
1	Cabbage	83.5 ± 3.0	90.0 ± 2.1	66.9 ± 2.4	32.6 ± 10.7	0.0 ± 2.6	5.3 ± 5.1	46.4
195	Sugar beet	8.3 ± 4.2	27.3 ± 3.2	99.0 ± 4.2	7.3 ± 4.6	30.0 ± 3.3	16.0 ± 9.3	31.3
184	Lobelia	56.7 ± 4.7	40.0 ± 3.7	32.1 ± 3.9	12.7 ± 3.4	2.7 ± 2.2	0.7 ± 2.7	24.2
193	Potato	35.0 ± 7.3	-9.3 ± 1.6	3.0 ± 3.6	66.0 ± 3.2	-4.0 ± 5.8	52.7 ± 2.7	23.9

nally isolated. The three most virulent conifer strains, however, gave somewhat greater differences than did the most virulent vegetable strain.

Turning attention to the results of inoculation tests on vegetable seedlings, it is apparent from the data in table 6 that greater variation existed in the susceptibility of the different species than was the case with the several conifer species. Pea, cabbage, and sugar beet were distinctly more susceptible than eggplant, pepper, and tomato.[3]

Of the conifer strains tested on vegetables, strain 187 was the most virulent on all species except pepper, on which none of the conifer strains was pathogenic. Strains 156 and 77 were not pathogenic to either pepper or tomato, while strain 167 did not give significant differences over the check in the case of eggplant, pepper, or tomato. Strains 160, 13, and 192 were pathogenic only to cabbage and pea. On the basis of these facts, together with that of the extreme differences in average results, it would appear that strain 187 was the most virulent on vegetables of any of the conifer strains tested, and that strains 13 and 192 were the least virulent.

Two of the strains from vegetables (196 and 183) were, with one exception, highly virulent on all species of vegetables tested. Strain 1 was highly virulent on cabbage, pea, and sugar beet, but was not pathogenic to eggplant, pepper, and tomato. Strains 195, 184, and 193 were not pathogenic to three suscepts each. The strains from cabbage, sugar beet, and pepper were the most virulent of any of the vegetable strains on the same species of vegetable from which they were respectively isolated. With one exception (strain 187 on sugar beet), the same is true even when all thirteen strains are considered.

From the tests with the thirteen strains on both conifers and vegetables, it would appear that strains 183 and 196 from vegetables were highly virulent on both groups of suscepts. Strain 187 from white pine was highly virulent on vegetables but not of great virulence on conifers. Strains 13, 77, 156, and 160 from conifers were highly virulent on conifers but of low virulence on vegetables. Strains 192 and 167 from conifers, and 193 and 195 from vegetables, were of generally low virulence on both groups of suscepts.

The foregoing relationship is illustrated diagrammatically in figure 1, but, since only the average figures from the last column in tables 5 and 6 are plotted, the diagram should be interpreted in the light of the preceding discussion.

Cultural studies

Among the numerous strains of Rhizoctonia isolated from conifers, it was observed that marked and more or less constant differences were maintained through successive transfers in tubes of potato-dextrose agar. This led to the study of a number of these strains in petri-dish cultures on cornmeal, oatmeal, potato, potato-dextrose, malt-extract, Czapek's, and

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[3] Tests were conducted also with potato. Severe infection occurred with strain 183, and slight infection with strain 193. In view of the extreme variation in soil moisture, the writer does not attach particular significance to the negative results obtained with the other strains.

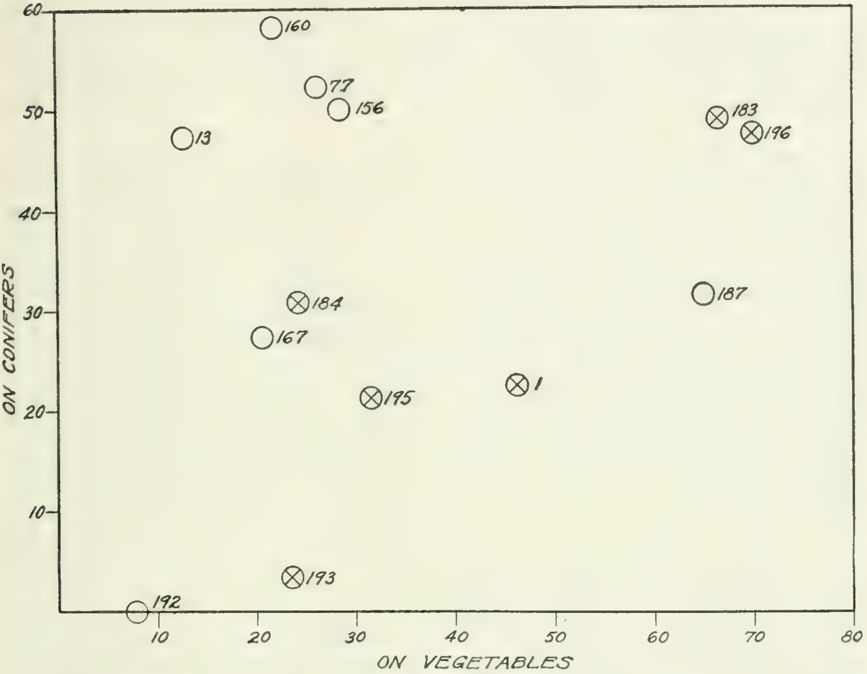

FIGURE 1. CROSS-INOCULATION TESTS WITH THIRTEEN STRAINS OF RHIZOCTONIA

The strains from conifers are designated by a plain circle, those from vegetables by a cross within a circle. The vertical scale represents the average difference between inoculated and check pots for each strain tested on conifers, this difference expressed in percentage of seed sown. The horizontal scale similarly represents the results of the tests on vegetable seedlings. (Data from last column of tables 5 and 6)

beef-peptone agars. Of these media, Czapek's, malt-extract, potato-dextrose, and cornmeal agars were selected as the standard media for later studies.[4] The twenty-three strains of Rhizoctonia already referred to

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[4] Formulae of the four media:

Czapek's synthetic-solution agar (Waksman, 1916, p. 113)

Distilled water	1000 cc.
$MgSO_4$	0.50 gm.
K_2HPO_4	1.00 gm.
KCl	0.50 gm.
$FeSO_4$	0.01 gm.
$NaNO_3$	2.00 gm.
Cane sugar	30.00 gm.
Agar	15.00 gm.

Malt-extract agar (Baxter, 1925, p. 528)

Distilled water	1000 cc.
Malt extract	25 gm.
Agar	25 gm.

Potato-dextrose agar

Distilled water	1000 cc.
Pared potatoes	200 gm.
Dextrose	10 gm.
Agar	15 gm.

Potatoes cooked until soft, and broth filtered through cheesecloth

Cornmeal agar

Distilled water	1000 cc.
Cornmeal	30 gm.
Agar	15 gm.

Maintained in water bath at 58° C. for one hour before adding agar

All media were boiled until the agar was dissolved, followed by a thirty-minutes heating in an Arnold sterilizer and slow cooling to permit settling and removal of sediment. Prior to pouring the plates, melting was accomplished by another thirty-minutes exposure to live steam. Determinations made by the electrometric method after the agars had finally solidified, indicated the following pH values for the different media: Czapek's, –6.82; potato-dextrose, –5.75; malt-extract, –4.87; cornmeal, 5.86.

were then grown in triplicate on each of these four media for five weeks at 25° C. Conspicuous differences between strains were evident on Czapek's agar, and to a lesser extent on malt-extract and potato-dextrose agars, while on cornmeal agar so little differentiation occurred that the cultures on that medium were discarded.

The four strains 77, 99, 141, and 177, which were selected as representing the majority of those isolated at the Keene nursery, were indistinguishable from one another on all three media. The principal characters of these strains are: on Czapek's agar, growth poor, sclerotia absent, the aerial hyphae tending to form irregular cottony tufts which are somewhat appressed to the surface of the agar; on malt-extract and potato-dextrose agars, growth abundant, presenting in general a buckthorn brown[5] color on the former and mummy brown on the latter; sclerotia formed on those two agars mostly less than one millimeter in diameter, indefinite in structure, for the most part only denser tufts of the fairly abundant aerial mycelium and profusely scattered over the agar; on malt-extract agar forming a few scattered sclerotia several millimeters in diameter, lighter in color than the mycelium and distinct from the aerial part of the latter (Plate II, 1–3).

A number of other strains (13, 84, 158, 160, 172, 190, 191, and 195) were similar to the above in many characters but differed from them and from one another in certain minor respects.

Several strains very distinct from all others encountered are represented in these studies by strain 156. The outstanding characters of this strain are: formation on Czapek's agar of numerous small compact sclerotia one millimeter or less in diameter and almost black in color, distinct or occasionally fused into oblong masses, always in striking contrast to the white or faintly brown, thin, opaque mycelial membrane; less abundant growth on malt-extract and potato-dextrose agars, with no evidence of a mycelial membrane and with sclerotia fewer and chiefly confined to the periphery of the agar, showing more pronounced fusion into masses on malt-extract agar (Plate II, 4–6).

An outstanding feature occurs in all of the other strains studied, namely, the formation on Czapek's agar of a very dense mycelial membrane on the surface of the medium. These strains vary widely, however, in other respects, as, for example, color, number, size, and type of sclerotia, and abundance of mycelium. Thus, strains 155 and 187 (Plate II, 10–12), which appear identical with the others, are characterized on the three agars by the nearly complete absence of conspicuous sclerotia and by the formation of a light-colored mycelium. Strains 167 and 175, which are believed to represent a considerable number of those isolated from conifers, are somewhat similar to strains 155 and 187 but differ in the formation on malt-extract and potato-dextrose agars of papery sclerotia which are light in color and are loosely fused with the abundant aerial mycelium (Plate II, 7–9). The four strains 1, 183, 184, and 193, on the contrary, form abundant distinct sclerotia (Plate III, 1–9). Strains 192 and 196 are intermediate in character between these four and the other four noted above (155, 187, 167, and 175) (Plate III, 10–12).

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[5] Colors according to Ridgway (1912).

Conclusions from the cross-inoculation and cultural studies

The most outstanding fact brought out by these experiments is that many of the strains of Rhizoctonia isolated from conifers differ strikingly in their virulence and in their morphological characters as expressed by growth on certain artificial media. Apparently the conifer strains differ as much from one another as they do from the vegetable strains. The morphological differences, however, are more striking than the differences in virulence noted, and are apparently not correlated with the latter.

In view of the fact that the cultural studies were of a somewhat limited nature, and since only gross morphological features have been considered in the grouping of the strains, it is not proposed to designate the groups with any particular name. With the possible exception of strain 156, which will be studied in more detail later, the strains employed are all considered to fall within the limits of *Rhizoctonia solani* Kühn as the species is now constituted.

CONTROL

The method of control most extensively tested was that of soil treatment with chemicals. A limited amount of information was obtained also on the effect of adding muck to the soil, and on the relation of fall seeding to the occurrence of the disease.

Application of muck

Because of the low content of organic matter in the soil at the Keene nursery, it has been the practice there to apply large quantities of muck every four or five years to the areas used for seedbeds. General observations by those in charge had suggested the possibility that such applications had been effective in the prevention of damping-off in certain parts of the nursery. Opportunity was afforded to make direct observations on seedbeds of norway spruce sown during the fall of 1926 and the spring of 1927 in a section of the nursery in which muck had never been used. The muck was added in November to several 100-foot beds as a layer several inches thick, after which it was harrowed into the soil. A part of this area was seeded in the fall, and the remainder during the following spring. On the fall-sown beds both with and without muck, approximately 30 per cent of the seedlings that emerged were destroyed by damping-off. More detailed observations were made on the spring-sown beds, where the following data were obtained from five nine-row plats in each of the treated and untreated areas.

Treatment	Percentage of emergence	Percentage of damping-off after emergence	Final stand
Treated with muck	8.2±0.6	57.2±2.7	148.8±12.9
Untreated	11.2±1.3	55.2±3.6	222.0±34.2

It is apparent that the results obtained with the muck were not significantly different from those where it was not used. Observations covering two years have failed to reveal any convincing evidence that the occurrence of damping-off in different parts of this nursery is associated with applications of muck to the soil.

Fall seeding

The practice of sowing coniferous seedbeds during the fall is followed by many nurserymen. Where practicable it has the advantage of distributing nursery operations more uniformly throughout the season, and thus relieving the congestion of work during the early spring months when transplanting and shipping operations are at their height. To carry out such a program, it is necessary to get the seed into the soil late enough to prevent fall germination and yet early enough to escape the colder weather which would make soil operations impossible. An obvious advantage of the practice is to insure early germination in the spring, and thus provide a longer growing season than would be available for spring-sown stock. Many nurserymen believe that the method has a further advantage in enabling them to avoid the losses from damping-off which are so common on beds sown during the spring, their reason being that by the time weather conditions are most favorable for damping-off the seedlings will have developed far enough to have escaped the disease.

Observations were made for one season, at the Keene nursery, on the relative abundance of the disease in beds sown during the fall of 1926 and in those sown at varying periods during the following spring. The several locations of the seedbeds were designated by letters. Locations B and C were in that part of the nursery where damping-off had been most severe in previous years. They were about 100 yards apart, and each in turn was several hundred yards removed from location A, where most of the beds were sown and where damping-off had never been a serious problem. The part of the nursery designated as location D was intermediate in position between B and C. In table 7 are given the percentages of damping-off that were recorded from the various beds.

TABLE 7. APPROXIMATE PERCENTAGE OF DAMPING-OFF AFTER EMERGENCE IN RELATION TO TIME OF SOWING AT THE KEENE NURSERY DURING FALL AND SPRING, 1926–27

Species sown	Location	November 4–6, 1926		April 1, 1927		April 12, 1927		April 21, 1927		May 6–7, 1927		May 12–16, 1927	
		Number of beds	Per cent	Number of beds	Per cent	Number of beds	Per cent	Number of beds	Per cent	Number of beds	Per cent	Number of beds	Per cent
Colorado spruce	A	20	16	17	5	...	...	8	15	...	...	...	....
Norway spruce	A	232	7	52	5	72	5	84	5	...	...	...	....
Norway spruce	B	20	30	...	...	...	...	...	...	60	58*	...	....
Norway spruce	D	20	20	...	...	...	...	...	...	...	...	...	....
Red pine	A	82	3	17	5	...	...	34	14	...	...	...	....
Red pine	D	9	15	...	...	...	...	...	...	...	...	...	....
Red pine	C	...	...	...	...	...	...	...	...	...	...	83	61*
White pine	A	109	5	...	...	...	...	83	5	...	...	...	....

* Average figure for check plats in soil-treatment experiments.

It is evident that with colorado spruce and white pine, losses were practically uniform on beds sown either in the spring or in the fall. The same is true of the beds of norway spruce at location A. At location B, however, losses were high in the fall-sown beds but were considerably lower than in those sown on May 6 and 7. High losses were noted also at location D, but it should be stated that these beds were considerably behind the other fall-sown beds in time of emergence. Losses on the red-pine beds at location A were greatest in those sown on April 21, but were just as high on the few fall-sown beds at location D. The fact that the most severe damping-off occurred in the beds of red pine and norway spruce which were sown in May does not necessarily indicate that the time of sowing was the chief factor involved, for, as already pointed out, these beds were situated in the sections of the nursery where the disease had been most prevalent in years past.

While the information obtained includes but one season's observations, the data as a whole indicate that damping-off may occur to a serious degree in fall-sown beds. The differences in prevalence of the disease at the nursery under discussion are not shown to be definitely related to the time of sowing.

Soil treatments with chemicals

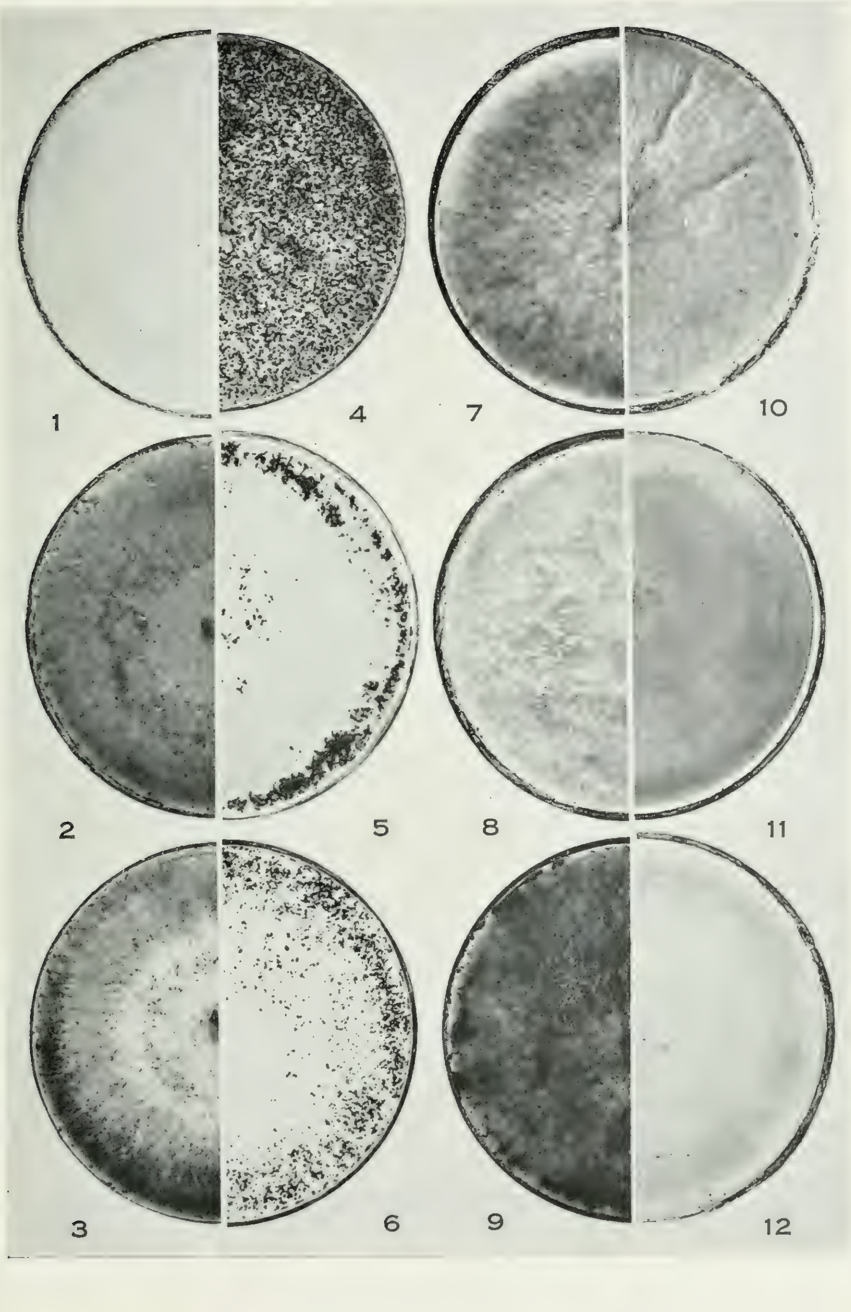
Field tests with chemicals were begun at the Ithaca nursery in 1925 and continued on a more extensive scale at the Keene nursery during the following two seasons.

The twelve beds of the Ithaca nursery were used for a single experiment. Each bed was divided into two sections 4 by 6 feet in size, thus providing for three checks and three replicates of seven different treatments. Chemicals were applied in solution, both directly after seeding and at various times for several weeks thereafter. Following seeding operations the beds were covered with burlap, which was removed as soon as emergence began and was allowed to remain on top of the half-shade frames for a

Plate II

CULTURES OF RHIZOCTONIA GROWN FOR FIVE WEEKS AT 25° C.

1, 2, and 3, Strain 77 on Czapek's, malt-extract, and potato-dextrose agars, respectively
4, 5, and 6, Strain 156 on Czapek's, malt-extract, and potato-dextrose agars, respectively
7, 8, and 9, Strain 167 on Czapek's, malt-extract, and potato-dextrose agars, respectively
10, 11, and 12, Strain 187 on Czapek's, malt-extract, and potato-dextrose agars, respectively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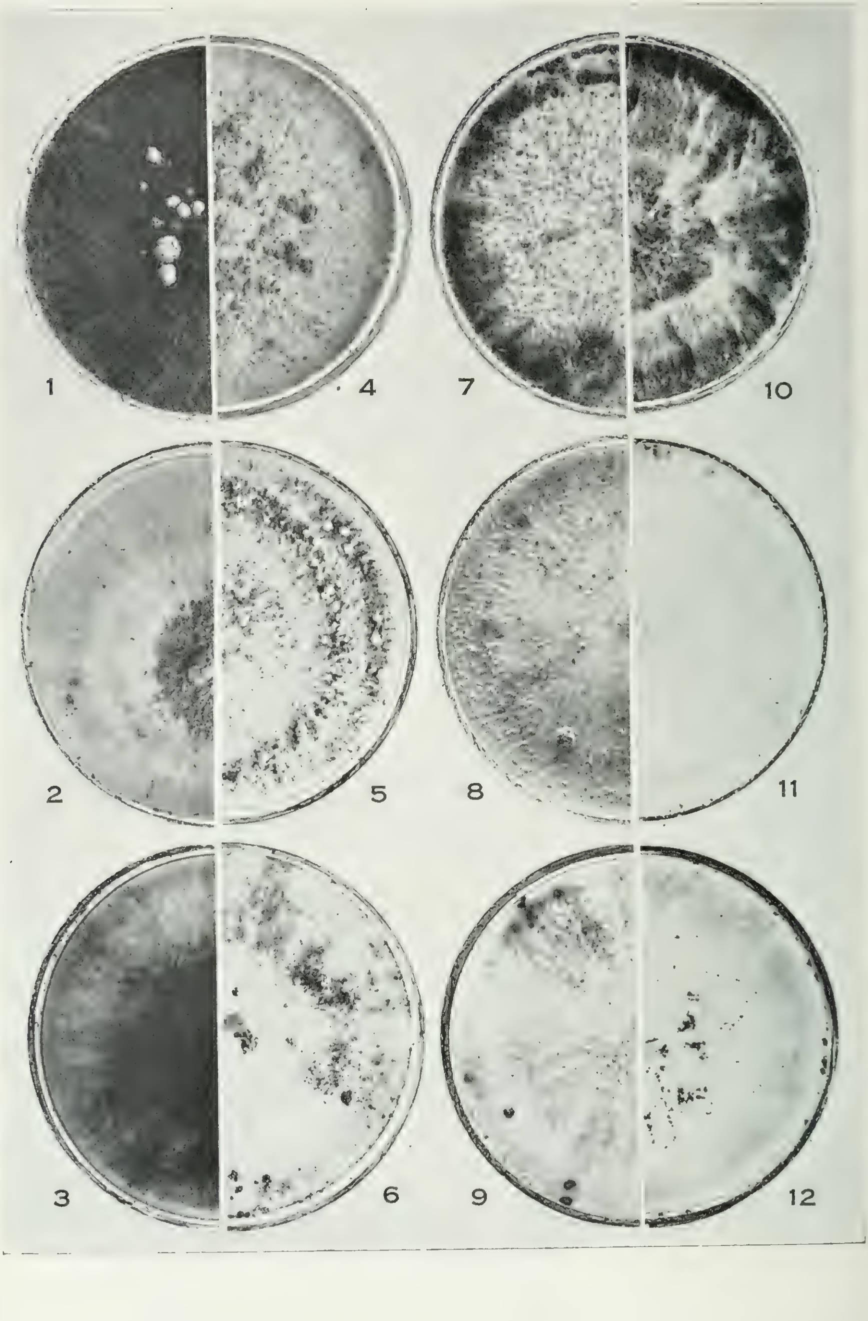
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Plate III

CULTURES OF RHIZOCTONIA GROWN FOR FIVE WEEKS AT 25° C.

1, 2, and 3, Strain 193 on Czapek's, malt-extract, and potato-dextrose agars, respectively
4, 5, and 6, Strain 183 on Czapek's, malt-extract, and potato-dextrose agars, respectively
7, 8, and 9, Strain 1 on Czapek's, malt-extract, and potato-dextrose agars, respectively
10, 11, and 12, Strain 192 on Czapek's, malt-extract, and potato-dextrose agars, respectively

period of four weeks. This additional shading, together with frequent sprinkling, maintained a rather high soil moisture and atmospheric humidity.

The experiments at the Keene nursery were conducted on an entirely different plan. Throughout both seasons it was attempted to conform in every possible way to the regular nursery practices in making up and caring for the beds. In order to provide for adequate replication of the various treatments, the beds were divided by board partitions into continuous series of small plats In the earliest experiments of 1926, these plats were 12 square feet in area and were sown with a definite quantity of seed distributed as evenly as possible in nine rows. For all later experiments the size of the plats was increased to 16 square feet, the number of rows remaining the same. The seed required for each set of three rows was weighed separately, thus providing for a more nearly uniform distribution than that afforded by seeding the entire nine rows from a single packet.

Liquid treatments were made from ordinary sprinkling cans directly after seeding. In some cases further treatments were made after emergence had occurred. Half of the check plats were sprinkled with an amount of water equal to that used in applying the chemicals to the treated plats. Where chemicals were applied in the dry condition, the materials were sifted on the plats from an envelope and worked into the upper inch of soil when the beds were given the final raking.

In the spring experiments of 1926, four replications were made of each treatment and a check plat was provided for every five to seven treated plats. In later tests, five replications were made and a single plat was left untreated for every five which were treated. The various replicates and checks were distributed over the beds as nearly uniformly as possible.

Materials

Aluminum sulfate, copper carbonate, mercuric chloride, mercurous chloride, sulfuric acid, and a number of commercial preparations containing organic compounds of mercury, were used at various times in the course of these investigations. The source and the grades of the different materials used in these experiments are given below. The descriptions of the composition and the chemical properties of the various commercial preparations are based upon statements of the manufacturers. The same is true for the changes in composition which have been made in several of the materials since the particular samples used in these tests were received.

Aluminum sulfate of technical grade was obtained from Merck & Co., of New York City, and a commercial grade was procured from the General Chemical Co., of New York City. By passing the pulverized form through sieves with twenty-five meshes to the inch, a fairly satisfactory material was obtained for use in the experiments with dry chemicals. This product was much coarser, however, than the mercury dusts.

The sulfuric acid employed was the commercial grade of concentrated acid, purchased in a local drug store.

Copper carbonate of technical grade was procured from the J. T. Baker Chemical Co., of Phillipsburg, New Jersey.

Mercuric chloride, U. S. P. IX, was obtained from Merck & Co., and mercurous chloride (calomel) from the Mallinckrodt Chemical Works, of New York City, under the trade name *Calogreen.* For the experiments with dry chemicals these two materials were diluted with talc.

The organic mercury preparations contained as their toxic ingredient either chlorophenol mercury (hydroxymercurichlorophenol sulfate), cyancresol mercury, or nitrophenol mercury (hydroxymercurinitrophenol sulfate). Of these preparations, Uspulun, Bayer compound, Bayer dust, and preparations I–8, II–6, 101, and 112, were furnished by the Bayer Company, of New York City. Their toxic constituents are as follows:

Name of preparation	Name and percentage of toxic constituents	Approximate solubility of the toxic constituents in water
		(Per cent)
Bayer compound	10% nitrophenol mercury	Complete
Bayer I–8	8% nitrophenol mercury	5
Bayer II–6	6% nitrophenol mercury	10
Bayer dust	4% nitrophenol mercury	5
Dipdust	2% nitrophenol mercury and 6% chlorophenol mercury	20
Bayer 112	3% nitrophenol mercury and 3% chlorophenol mercury	5
Bayer 101	10% chlorophenol mercury	80
Uspulun	30% chlorophenol mercury	Complete

Semesan Bel, Semesan, and Semesan Jr., obtained from E. I. du Pont de Nemours & Co., of Wilmington, Delaware, contained respectively 10 per cent, 35 per cent, and 10 per cent of chlorophenol mercury. This chemical as it occurred in Semesan was almost completely soluble in water, in Semesan Bel it was only slowly soluble, and in Semesan Jr. approximately one-third was immediately soluble.[6]

Germisan, containing approximately 31 per cent of cyancresol mercury, was obtained from the Saccharin-Fabrik, Aktiengesellschaft, Magdeburg Südost, Germany.

The mercury content of chlorophenol mercury is 61 to 62 per cent, in comparison with 55 per cent in nitrophenol mercury, 73.89 per cent in mercuric chloride, and 84.96 per cent in mercurous chloride.

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[6] Several changes have been made in the composition of two of these preparations since the experiments here reported were conducted. Thus, as manufactured in June, 1928, Semesan Bel contained the chlorophenol mercury in a form which was about 40 per cent immediately soluble, while Semesan Jr. contained as the toxic constituent 12 per cent of cresol mercury, approximately 40 per cent of which was readily soluble.

Methods of taking and interpreting data

The methods employed in taking data on damping-off were varied somewhat from season to season, depending largely upon the stage of seedling development at which the disease first appeared. Thus, in the spring and early summer experiments of 1926, emergence in the beds of norway spruce and red pine was practically complete before more than a trace of damping-off had appeared. Counts of all seedlings in three rows in each plat were made on such beds as soon as emergence had taken place. Counts were again taken of the final stand in the same rows in early September. Observations made during the intervening time indicated that the differences between the two sets of figures represented losses caused by the disease. Damping-off appeared in beds of white pine soon after the first seedlings had pushed through the soil, and continued during and after the emergence period. Because of this, a single count of the final stand was made in Sepetmber, on four rows in each plat.

During the season of 1927, losses from damping-off were evident in the beds of norway spruce and red pine from the very beginning of the emergence period. This fact, combined with the loss of a number of seedlings by bird injury,[7] made frequent counts necessary. All plats were therefore inspected at short intervals to record and remove from four rows in each plat all seedlings which were damped-off and the stubs of seedlings which had been broken off by birds. As soon as emergence was over, the surviving seedlings were counted, and this was followed by another count at the end of the season. The difference between the two indicated the loss from damping-off during this period. Where losses from disease were slight throughout the season, it frequently happened that the number damped-off was more than equaled by the number of late-emerging seedlings which always appear in conifer seedbeds even many weeks after the vast majority of seedlings have emerged. These differences are indicated as plus figures under the heading of percentage of damping-off, as in the 1926 experiments. In all cases they are small and are entirely without significance.

In the beds of white pine, damping-off likewise appeared during the general emergence period. The more severe bird injury in these beds made it desirable to count only the final stand at the close of the season. It is pointed out later in this work that the results of these experiments on white pine cannot be accorded the same significance as those with red pine and norway spruce.

As a result of the unequal distribution of the losses resulting from bird injury, a sound interpretation of the data obtained from the norway-spruce and red-pine experiments of 1927 necessarily involved the introduction of a correcting factor. This was done in the following manner.

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[7] When conifer seedlings first emerge from the seedbeds, the immature cotyledons still inclosed within the seed coat are borne at the tip of the hypocotyl. The type of bird injury referred to was occasioned by the action of sparrows and goldfinches in feeding on these tips. As a result, only the broken hypocotyl remained. The danger from bird injury existed only until the cotyledons were mature and the seed coats shed.

For each plat the total loss from bird injury was subtracted from the total emergence. Losses from damping-off were then based upon this remainder, which represented the total number of seedlings surviving bird injury and from which the percentage of damping-off was calculated. It was then assumed that the seedlings which were destroyed by birds would have damped-off at the same rate as those which were not destroyed. The final stand as indicated in the various tables therefore represents the total emergence minus the sum of the actual damping-off and the theoretical damping-off estimated for the seedlings removed by bird injury. The data on final stand are given on the basis of nine rows in each plat.

Essentially the same method was employed in interpreting the data from the Ithaca experiment of 1925, there having been a rather uneven occurrence of losses due to mechanical breakage.

The figures for each set of replicate plats were averaged and the probable error of this mean was determined by the use of Bessel's formula. The same was done for the check plats of each experiment. In view of the rather limited number of replications employed, differences between any two means are not considered significant unless they have at least four times the value of the probable error of this difference.

Results of the soil-treatment experiments

The results obtained from the soil treatments are presented according to the species of conifer sown in the experimental plats.

Red pine — Liquid treatments. Preliminary field tests with sulfuric acid and several mercury disinfectants were made on beds of red pine at the Ithaca nursery in 1925. The beds were artificially infested with a damping-off fungus (Rhizoctonia) and were maintained under rather wet conditions. The incidence of disease was very high (table 8). Thus in the untreated

TABLE 8. LIQUID TREATMENTS ON BEDS OF RED PINE SOWN AT THE ITHACA NURSERY ON MAY 15, 1925

Material employed	Number of applications	Amount per square foot	Results (average of three replications)		
			Percentage of emergence	Percentage of damping-off after emergence	Final stand[††]
Bayer compound	5	2.58 gms.[*]	30.0±2.1	43.3± 4.8	260±29.9
Germisan	2	1.33 gms.[†]	....	....	....
Mercuric chloride	5	1.03 gms.[‡]	46.0±1.0	22.0± 3.5	536±61.2
Sulfuric acid	1	5 cc.[§]	36.0±3.3	61.7±10.6	230±67.3
Uspulun	2	3.01 gms.[‖]	39.7±1.8	24.3± 3.5	449±33.0
Uspulun	3	3.34 gms.[¶]	28.7±1.4	28.0± 2.1	307±17.1
Uspulun	6	4.02 gms.[**]	35.3±5.9	19.7± 3.6	442±85.7
Check (untreated)	......	....	25.7±3.9	98.0± 0.8	8± 3.1

[*] 0.7 gram in 400 cc. water at time of seeding; 0.47 gram in 270 cc. four, six, eight, and ten weeks later.

[†] 0.8 gram in 400 cc. water at time of seeding; 0.53 gram in 270 cc. four weeks later.

[‡] 0.33 gram in 400 cc. water at time of seeding; 0.22 gram in 270 cc. four weeks later; 0.16 gram in 270 cc. six, eight, and ten weeks after seeding.

[§] Equivalent to from 5/32 to 6/32 fluid ounce of concentrated acid dissolved in 750 cc. water and applied at time of seeding.

[‖] 2.34 grams in 940 cc. water at time of seeding; 0.67 gram in 270 cc. four weeks later.

[¶] 2.0 grams in 800 cc. water at time of seeding; 0.67 gram in 270 cc. four and six weeks later.

[**] 0.67 gram in 270 cc. water at time of seeding, and repeated two, four, six, eight, and ten weeks later.

[††] A total of 1500 seeds were sown in the area of each section from which data were obtained.

beds 98 per cent of the seedlings were destroyed, the greater part of the loss occurring during the first week after emergence began. Treatments with mercuric chloride and Uspulun caused a considerable reduction in damping-off and a marked increase in final stand. So many seedlings were killed by the second application of Germisan that these beds were discarded. Bayer compound and sulfuric acid were distinctly less effective in controlling the disease than were the other chemicals. While the experiment was carried out on a small scale and under rather artificial conditions, the results indicated that several of the materials were promising for future tests.

In 1926 a more extensive experiment was conducted with this species at the Keene nursery, solutions of aluminum sulfate, sulfuric acid, and three mercury fungicides, being used. Damping-off did not appear to any appreciable extent until emergence was nearly complete.

With the exception of the two weakest applications of sulfuric acid, and treatments 12 and 18 with Bayer compound and mercuric chloride, respectively, significant differences in percentage of loss after emergence occurred in all cases (table 9).

Because of the low incidence of disease in the check plats and the variation within any group of replicates, significant differences in final stand between treated and untreated plats did not occur with treatments 2, 5, 12, 15, 17, 18, 19, and 20.

Slight stunting was noted in several plats treated with the strongest sulfuric-acid solution, and in those plats where second applications of mercuric chloride were made. However, observations made at the end of the second growing season failed to reveal any difference in the size or the appearance of the seedlings on treated and untreated plats. Evidently those seedlings surviving the second application of mercuric chloride were not permanently stunted.

The experiment showed that certain concentrations of all of the fungicides were effective in controlling damping-off, even when applied only at seeding time, and that such treatments did not cause chemical injury.

Three experiments were conducted with liquid treatments on red pine during the season of 1927. Experiment C was sown on April 21 at the rate of 2700 seeds in each plat, while experiments A and B were sown on May 12 and May 16, respectively, at the rate of 1800 seeds. The results for the three experiments are given in table 10. The percentage of emergence was in all cases somewhat higher in the treated plats. In experiments A and B, 60.3 per cent and 38.7 per cent, respectively, of the seedlings which emerged in the checks were destroyed by damping-off, while all treatments reduced the disease to a negligible amount. The final stand for all treatments of experiments A and B was very high, and in all cases was significantly greater than that of the check. The differences between the final stands of the several treatments are, however, not significant.

TABLE 9. LIQUID TREATMENTS ON BEDS OF RED PINE SOWN AT THE KEENE NURSERY ON MAY 26, 1926

Treatment no.	Material employed	Rate of application per square foot				Number of replications	Results		
		At seeding time		Two months after seeding					
		Weight of material	Quantity of water in which applied	Weight of material	Quantity of water in which applied		Percentage of emergence	Percentage of loss after emergence	Final stand per 9-row plat
			(Cubic centimeters)		(Cubic centimeters				
1	Aluminum sulfate	7.087 gms.	500	....	....	4	72.0 ± 3.1	6.8 ± 2.2	1,876 ± 74.6
2		10.631 gms.	500	....	....	4	67.0 ± 4.4	3.3 ± 0.8	1,796 ± 107.8
3		14.175 gms.	500	....	....	3	71.0 ± 0.7	1.3 ± 1.6	1,959 ± 20.5
4		17.718 gms.	500	....	....	3	72.3 ± 1.8	4.0 ± 2.2	1,943 ± 11.7
5	Sulfuric acid	2/32 fl. oz.	500	....	....	4	63.3 ± 2.9	14.8 ± 4.5	1,491 ± 59.2
6		3/32 fl. oz.	500	....	....	3	70.3 ± 2.7	10.7 ± 4.3	1,744 ± 19.9
7		4/32 fl. oz.	500	....	....	3	62.7 ± 1.4	7.3 ± 2.4	1,621 ± 9.1
8		5/32 fl. oz.	500	....	....	3	66.3 ± 1.1	1.7 ± 1.9	1,822 ± 10.0
9		6/32 fl. oz.	500	....	....	4	66.5 ± 2.0	2.0 ± 0.5	1,824 ± 62.6
10	Bayer compound	2.625 gms.	750	....	....	4	69.5 ± 2.2	7.8 ± 2.3	1,780 ± 19.5
11		1.875 gms.	750	....	....	4	68.0 ± 1.5	10.3 ± 1.6	1,715 ± 54.4
12		1.875 gms.	750	1.25 gms.	500	4	70.3 ± 1.6	9.8 ± 4.1	1,782 ± 111.4
13		1.250 gms.	500	....	....	4	72.3 ± 1.8	4.3 ± 1.0	1,941 ± 64.6
14		1.250 gms.	500	0.625 gm.	250	4	70.0 ± 2.2	4.3 ± 1.0	1,870 ± 64.7
15*	Mercuric chloride	0.6206 gm.	750	....	....	4	63.0 ± 1.2	6.5 ± 2.0	1,642 ± 30.6
16		0.4433 gm.	750	....	....	3	63.7 ± 0.8	3.0 ± 1.4	1,726 ± 13.8
17		0.4433 gm.	750	0.2955 gm.	500	3	66.3 ± 2.6	10.3 ± 1.8	1,667 ± 54.1
18		0.2955 gm.	500	....	....	3	69.7 ± 2.9	11.7 ± 5.4	1,744 ± 171.7
19		0.2955 gm.	500	0.1478 gm.	250	4	67.5 ± 1.0	12.0 ± 3.1	1,669 ± 70.5
20*	Uspulun	2.625 gms.	750	....	....	3	69.0 ± 2.9	8.7 ± 0.2	1,760 ± 80.9
21		1.875 gms.	750	....	....	3	68.7 ± 0.2	5.0 ± 1.8	1,826 ± 31.0
22		1.875 gms.	750	1.250 gms.	500	4	69.5 ± 2.0	8.5 ± 2.7	1,786 ± 82.9
23		1.250 gms.	500	....	....	4	70.3 ± 1.4	6.0 ± 2.5	1,856 ± 74.1
24		1.250 gms.	500	0.625 gm.	250	4	76.0 ± 2.2	8.5 ± 2.4	1,948 ± 55.4
	Check (untreated)	....	....	....	....	12	69.9 ± 1.4	30.8 ± 3.5	1,350 ± 67.4

* Treatments 15 and 20 are equivalent in mercury content, as are also treatments 16 and 21, 17 and 22, 18 and 23, and 19 and 24.

TABLE 10. LIQUID TREATMENTS ON BEDS OF RED PINE AT THE KEENE NURSERY, SEASON OF 1927

	Treatment no.	Treatment		Number of replications	Results		
		Material employed	Amount per square foot applied in 500 cc. of water		Percentage of emergence	Percentage of damping-off after emergence	Final stand per 9-row plat
Experiment A	1	Aluminum sulfate	10.631 gms.	5	81.2 ± 1.8	6.8 ± 2.9	1,369 ± 61.0
	2		14.175 gms.	4	79.8 ± 1.5	1.5 ± 0.6	1,413 ± 33.1
	3		17.718 gms.	5	77.4 ± 1.9	1.0 ± 0.7	1,367 ± 35.4
	4	Sulfuric acid	4/32 fl. oz.	5	79.2 ± 1.1	7.8 ± 2.0	1,313 ± 42.8
	5		5/32 fl. oz.	5	74.2 ± 1.1	8.0 ± 2.4	1,231 ± 31.3
	6		6/32 fl. oz.	5	78.2 ± 2.4	3.4 ± 0.3	1,351 ± 40.9
	7*	Mercuric chloride	0.1182 gm.	6	76.0 ± 1.9	0.3 ± 1.5	1,337 ± 34.2
	8		0.2069 gm.	6	73.7 ± 2.6	+1.0 ± 1.3	1,312 ± 44.7
	9	Semesan	0.500 gm.	6	75.3 ± 2.8	3.2 ± 2.7	1,292 ± 49.9
	10		0.875 gm.	6	77.0 ± 2.6	0.8 ± 1.0	1,355 ± 43.9
	11*	Uspulun	0.500 gm.	6	76.2 ± 3.0	4.8 ± 3.2	1,305 ± 82.0
	12		0.875 gm.	6	74.2 ± 1.8	+1.3 ± 1.0	1,325 ± 34.5
		Check (untreated)	....	15	64.5 ± 2.1	60.3 ± 5.5	514 ± 77.6
Experiment B	1	Mercuric chloride	0.2364 gm.	6	74.0 ± 1.8	+0.2 ± 0.4	1,321 ± 32.6
	2		0.4137 gm.	6	71.8 ± 1.2	+0.3 ± 0.3	1,292 ± 22.3
	3	Semesan	1.00 gm.	6	73.7 ± 2.5	+0.3 ± 1.3	1,303 ± 40.2
	4		1.75 gms.	6	70.8 ± 2.3	2.2 ± 1.3	1,234 ± 27.1
	5	Uspulun	1.00 gm.	5	75.8 ± 1.4	0.3 ± 0.8	1,351 ± 24.2
	6		1.75 gms.	6	71.8 ± 1.1	+0.7 ± 0.6	1,283 ± 22.5
		Check (untreated)	....	7	66.9 ± 2.9	38.7 ± 7.1	780 ± 106.8
Experiment C	1	Aluminum sulfate	10.631 gms.	3	66.7 ± 2.1	2.7 ± 1.2	1,739 ± 35.5
	2		17.718 gms.	3	64.7 ± 2.4	6.7 ± 3.0	1,618 ± 55.8
	3	Sulfuric acid	4/32 fl. oz.	3	58.0 ± 3.4	20.3 ± 11.1	1,203 ± 120.9
	4		6/32 fl. oz.	3	60.3 ± 0.9	11.7 ± 2.6	1,445 ± 24.3
		Check (untreated)	....	5	55.2 ± 4.6	48.0 ± 4.4	811 ± 127.4

* Treatments 7 and 11, and 8 and 12, of experiment A are equivalent in mercury content, as are also treatments 1 and 5, and 2 and 6, of experiment B.

It is apparent, then, that the liquid treatments were equally effective in controlling damping-off, even where very small amounts of the mercury materials were used and under conditions in which severe damping-off occurred in the check plats (figure 2). With the exception of an occasional group of slightly stunted seedlings in several plats treated with the stronger applications, no evidence of chemical injury could be detected (Plate IV, 1 and 2, and Plate V, 1).

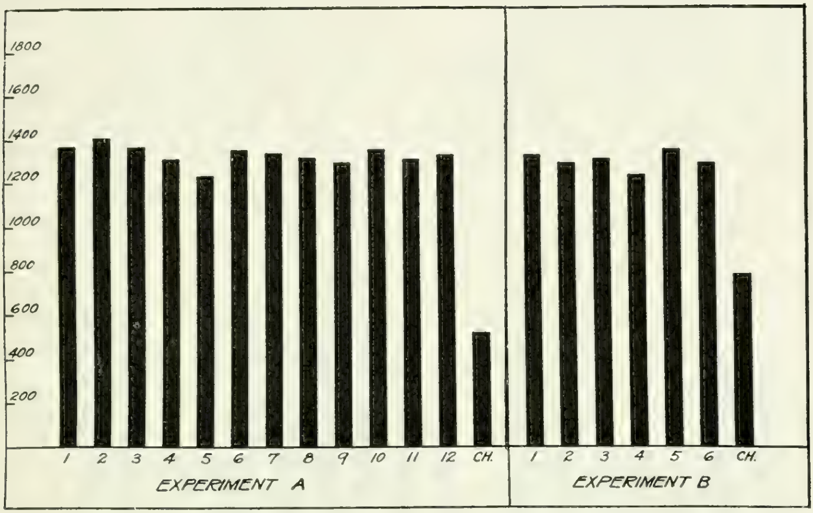


FIGURE 2. FINAL STAND OF RED-PINE SEEDLINGS IN PLATS TREATED WITH LIQUID APPLICATIONS OF CHEMICALS

The numbers below the columns refer to the treatment numbers given in table 10

In experiment C, sulfuric acid was less effective than aluminum sulfate in controlling the disease. The results with the weaker acid treatment were not significantly different from those obtained from the check plats.

Red pine — Treatments with dry chemicals. Preliminary experiments were conducted at the Keene nursery in 1926 with applications of finely divided copper carbonate and Bayer dusts I–8, 101, and 112, at the rate of 4 and 8 grams per square foot. Favorable results were obtained on a small scale with the mercury dust, while the copper carbonate caused severe injury. Bayer dust, Dipdust, and copper carbonate were similarly used on fall-sown beds of the same year. The copper carbonate (1, 2, and 3 grams per square foot) did not control the disease in the smaller amounts and caused injury in the larger applications. The mercury dusts (3 and 6 grams per square foot) were only partially effective against the disease.

Spring applications of dusts made directly on the surface of fall-sown plats just prior to emergence, were approximately as effective as the fall treatments. Dusting the seed alone was practically without effect.

An experiment was conducted on red pine during the season of 1927 with dry applications of aluminum sulfate and a number of organic and inorganic mercury materials (table 11). The experiment was comparable to experiment A of the liquid treatments of the same season.

Many of the mercury treatments were effective in reducing the disease from 67.7 per cent, as noted for the checks, to an almost negligible amount. From a consideration of the percentage of damping-off and the final stand (figure 3), it would appear that the best results were obtained with the two treatments with Bayer II–6, the heavier application of mercuric chloride, and the two heaviest applications of mercurous chloride and of Semesan Jr. The 2-gram applications of Bayer dust and of Dipdust were, on the other hand, rather ineffective in controlling the disease. Of the remaining treatments, those with the two heaviest applications of aluminum sulfate, the 4-gram application of Bayer dust, and the 4- and 6-gram applications of Dipdust, gave the least satisfactory results (Plate V, 2).

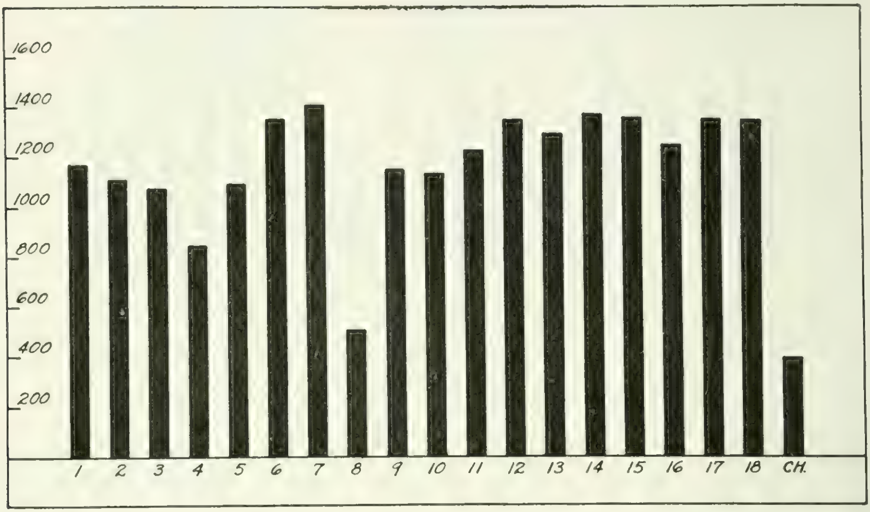


FIGURE 3. FINAL STAND OF RED-PINE SEEDLINGS IN PLATS TREATED WITH DRY APPLICATIONS OF CHEMICALS

The numbers below the columns refer to the treatment numbers given in table 11

No evidence of chemical injury was noted except in a few of the plats receiving treatments 11, 12, 14, 15, and 18, where there was an indication

TABLE 11. TREATMENTS WITH DRY CHEMICALS ON BEDS OF RED PINE AT THE KEENE NURSERY, SEASON OF 1927

Treatment no.	Treatment		Number of replications	Results		
	Material employed	Amount applied per square foot		Percentage of emergence‡	Percentage of damping-off after emergence	Final stand per 9-row plat
1	Aluminum sulfate	7.087 gms.	5	72.8 ± 1.4	10.8 ± 2.3	1,168 ± 51.3
2		10.631 gms.	5	71.0 ± 2.7	13.2 ± 1.7	1,110 ± 56.7
3		14.175 gms.	4	73.0 ± 1.7	17.0 ± 5.5	1,079 ± 48.6
4	Bayer dust	2 gms.	5	70.8 ± 2.8	33.2 ± 3.6	846 ± 43.5
5		4 gms.	5	78.6 ± 2.4	23.2 ± 3.6	1,099 ± 79.7
6	Bayer II–6	4 gms.	4	77.5 ± 0.8	3.3 ± 0.7	1,355 ± 12.3
7		6 gms.	5	80.4 ± 2.9	2.4 ± 0.7	1,410 ± 51.8
8	Dipdust	2 gms.	4	65.8 ± 5.4	58.8 ± 6.5	510 ± 104.4
9		4 gms.	5	81.4 ± 2.5	22.2 ± 4.4	1,153 ± 91.1
10		6 gms.	5	71.0 ± 1.6	11.6 ± 1.9	1,131 ± 41.7
11*	Mercuric chloride	0.2364 gm.	5	72.6 ± 2.2	6.4 ± 1.7	1,228 ± 50.1
12		0.4137 gm.	4	78.5 ± 2.5	4.0 ± 1.2	1,351 ± 37.6
13*	Mercurous chloride	0.2056 gm.	5	75.2 ± 0.8	4.0 ± 1.4	1,297 ± 25.7
14		0.3598 gm.	5	79.2 ± 1.4	3.2 ± 1.0	1,376 ± 28.8
15		0.3598 gm.†	5	76.2 ± 3.1	0.6 ± 0.5	1,360 ± 53.1
16	Semesan Jr.	2 gms.	5	80.8 ± 3.1	13.8 ± 4.1	1,246 ± 73.4
17		4 gms.	5	79.0 ± 1.3	4.6 ± 1.3	1,354 ± 30.3
18		6 gms.	5	76.8 ± 0.7	2.6 ± 0.6	1,345 ± 13.7
	Check (untreated)	....	19	59.6 ± 2.1	67.7 ± 3.6	397 ± 51.9

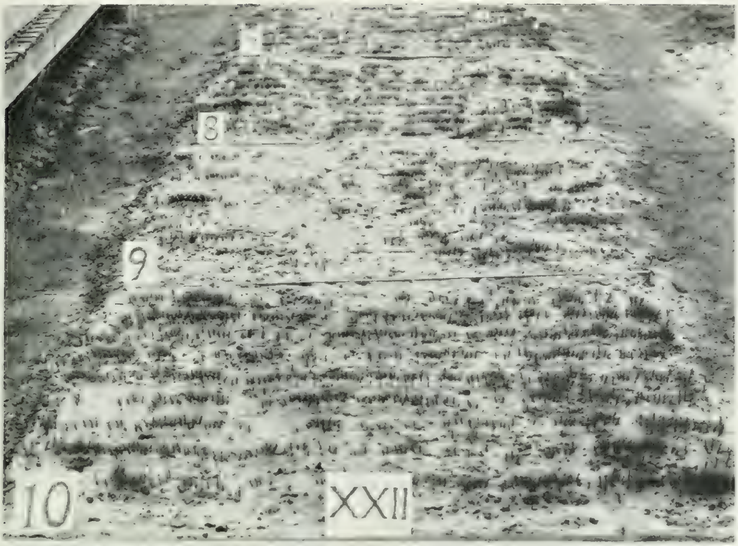
* Treatments 11 and 13 are equivalent in mercury content, as are also treatments 12, 14, and 15.
† Applied in 250 cc. water immediately after seeding.
‡ Sown at the rate of 1800 seeds in each plat.

PLATE IV

FINAL STAND OF RED-PINE SEEDLINGS IN BEDS RECEIVING LIQUID TREATMENTS FOR CONTROL OF DAMPING-OFF

1: Plat 9, untreated; plat 7, 0.875 gram of Semesan per square foot; plat 8, 0.1182 gram of mercuric chloride per square foot; plat 10, 0.2069 gram of mercuric chloride per square foot

2: Plat 15, untreated; plat 13, 0.875 gram of Uspulun per square foot; plat 14, 0.500 gram of Semesan per square foot; plat 16, 4/32 fluid ounce of sulfuric acid per square foot



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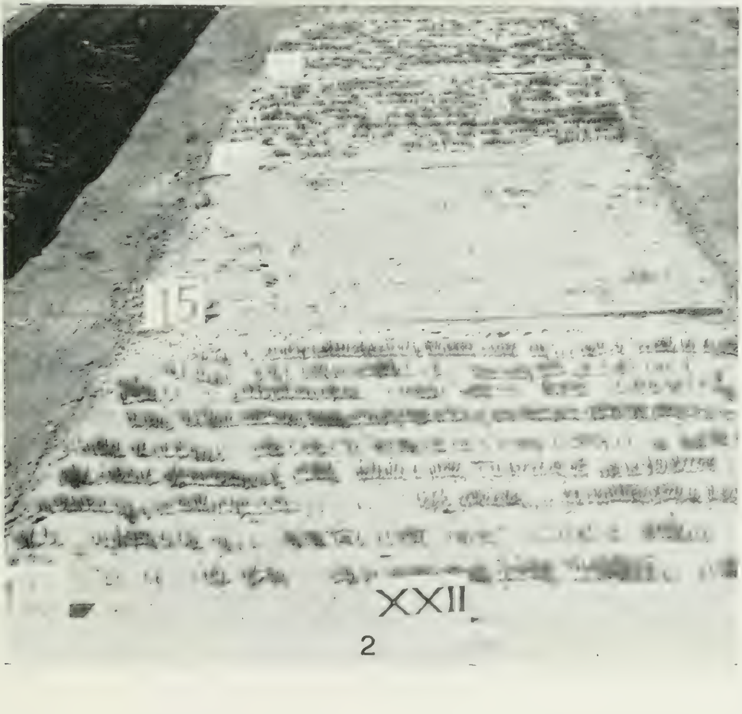

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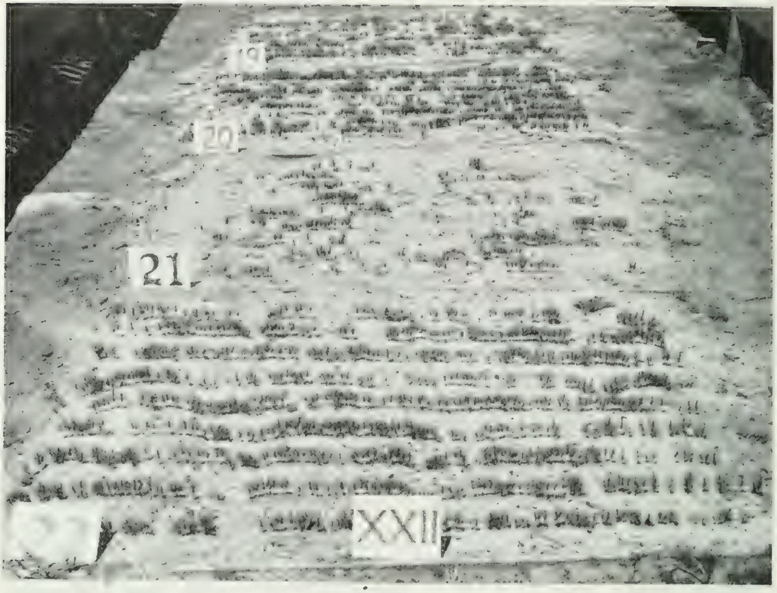

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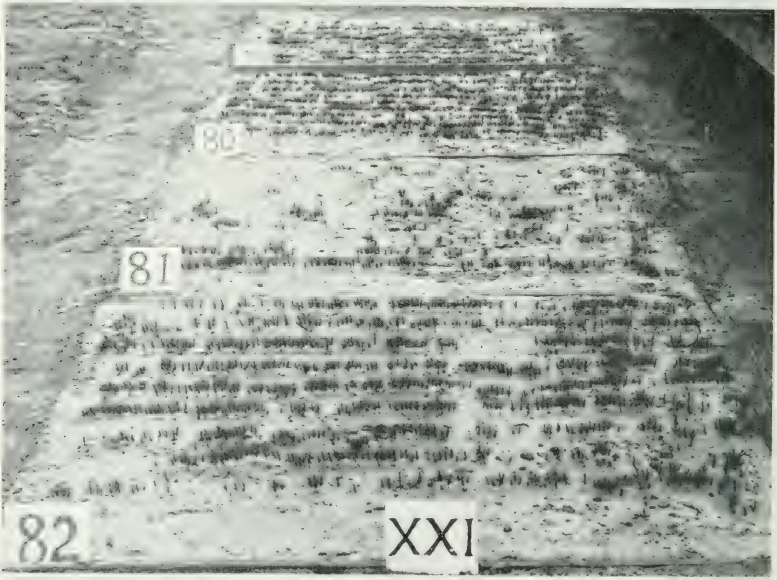

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Plate V

FINAL STAND OF RED-PINE SEEDLINGS IN BEDS TREATED WITH CHEMICALS FOR CONTROL OF DAMPING-OFF

1: Liquid treatments: Plat 21, untreated; plat 19, 3/8 ounce of aluminum sulfate per square foot; plat 20, 4/8 ounce of aluminum sulfate per square foot; plat 22, 0.875 gram of uspulun per square foot

2: Treatments with dry chemicals: Plat 81, untreated; plat 79, 0.4137 gram of mercuric chloride per square foot; plat 80, 0.2056 gram of mercurous chloride per square foot; plat 82, 0.3598 gram of mercurous chloride per square foot of very slight stunting. This injury is of no particular significance except to indicate the maximum amount of these chemicals which can safely be employed under these conditions.

Norway spruce — Liquid treatments. Solutions of mercuric chloride and several organic mercury materials were used on spring-sown beds of norway spruce during the season of 1926. The details of the experiment are recorded in table 12. The low figures for percentage of emergence in both the treated and the untreated plats are due in part to the low viability of the seed and in part to the fact that the seeds were sown at a greater depth than usual. Damping-off, which occurred chiefly in the check plats, was rather unevenly distributed. The heavy losses noted from treatments 2, 8, 10, and 12 were due primarily to the injurious effects of the second application of chemical. Although the losses with the other treatments were considerably lower than those of the check, significant differences occurred only with treatments 1, 3, 6, 13, 15, 17, 21, and 23. Similarly, for the final stand significant differences were found only with treatments 11, 15, and 23.

Second applications of mercuric chloride and of the stronger treatment with Bayer compound caused marked injury. This was manifested by a killing of some seedlings and a severe stunting of others. The larger amounts of the other chemicals caused slight retardation, while with the smaller amounts slight stunting was noted in several of the replicates. With the single-application treatments, retardation was noted in several plats where the larger amounts were used. Observations were again made at the end of the second growing season. Although considerable variation in the size of the seedlings occurred, it was possible to group the plats in a general way. In the plats of class A, therefore, most of the seedlings were typical of the average stock commonly used for transplanting. In those of class B the stock was of a somewhat smaller size than in A, but large numbers of these seedlings would normally be transplanted. In those of class C the majority of the seedlings were of decidedly inferior quality and would be discarded.

This experiment indicates that norway spruce is rather susceptible to chemical injury, and that of the treatments employed only the single applications with the smaller amounts are practicable for use on this species under these conditions.

Liquid treatments were used also in one series of norway-spruce plats sown in the fall of 1926. So little damping-off occurred that counts were made only of the final stand. From table 13 it is seen that stunting occurred from a number of the treatments. Since germination did not take place until the spring of 1927, five months after the time of treating, it would appear that either the chemicals exerted a toxic action upon the dormant seed, or else they were present in a readily available state at the time of germination. The latter hypothesis seems the more tenable.

TABLE 12. LIQUID TREATMENTS ON BEDS OF NORWAY SPRUCE SOWN AT THE KEENE NURSERY ON MAY 21, 1926

Treatment no.	Material employed	Treatment				Number of replications	Results			
		Rate of application per square foot								
		At seeding time		On July 8						
		Weight of material	Quantity of water in which applied	Weight of material	Quantity of water in which applied		Percentage of emergence†	Percentage of loss after emergence	Final stand per 9-row plat	Classification of plats
			(Cubic centimeters)		(Cubic centimeters)					
1	Bayer compound	1.250 gms.	500	0.625 gm.	250	4	53.5 ± 0.2	+1.3 ± 2.9	1,680 ± 42.3	A
2		1.750 gms.	500	0.875 gm.	250	4	52.8 ± 1.5	9.3 ± 2.9	1,478 ± 22.9	A
3		1.875 gms.	750	....	....	4	43.5 ± 4.8	+2.8 ± 3.3	1,387 ± 170.8	A
4		2.625 gms.	750	....	....	4	50.0 ± 1.8	3.5 ± 3.4	1,496 ± 79.0	A
5*	Mercuric chloride	0.2955 gm.	500	....	....	4	53.5 ± 1.5	2.8 ± 2.6	1,607 ± 26.5	A
6		0.2955 gm.	500	0.1478 gm.	250	4	54.8 ± 1.0	2.8 ± 0.7	1,636 ± 33.3	A
7		0.4137 gm.	500	....	....	3	53.8 ± 2.0	4.0 ± 1.5	1,533 ± 60.2	A
8		0.4137 gm.	500	0.2069 gm.	250	4	56.0 ± 2.7	21.3 ± 2.0	1,362 ± 69.5	C
9		0.4433 gm.	750	....	....	4	56.5 ± 2.6	7.5 ± 2.1	1,606 ± 59.2	A
10		0.4433 gm.	750	0.2955 gm.	500	4	56.0 ± 1.1	11.0 ± 3.0	1,551 ± 74.8	B
11		0.6206 gm.	750	....	....	3	54.5 ± 2.1	1.3 ± 2.8	1,720 ± 41.7	B
12		0.6206 gm.	750	0.4137 gm.	250	3	53.0 ± 0.5	21.0 ± 3.3	1,312 ± 59.4	C
13	Semesan	1.250 gms.	500	0.625 gm.	250	4	58.0 ± 2.6	+2.8 ± 2.8	1,855 ± 115.5	A
14		1.750 gms.	500	0.875 gm.	250	3	56.3 ± 0.7	4.3 ± 2.0	1,658 ± 54.9	A
15		1.875 gms.	750	....	....	4	55.8 ± 0.7	+4.8 ± 4.7	1,805 ± 58.2	A
16		2.625 gms.	750	....	....	4	52.0 ± 1.1	3.8 ± 1.8	1,545 ± 16.9	B
17*	Uspulun	1.250 gms.	500	....	....	4	52.8 ± 0.7	0.8 ± 0.9	1,626 ± 34.2	A
18		1.250 gms.	500	0.625 gm.	250	4	50.0 ± 4.4	4.8 ± 1.9	1,477 ± 128.2	A
19		1.750 gms.	500	....	....	4	53.3 ± 2.2	2.5 ± 1.8	1,612 ± 59.3	B
20		1.750 gms.	500	0.875 gm.	250	4	55.3 ± 2.6	4.0 ± 2.2	1,650 ± 86.6	B
21		1.875 gms.	750	....	....	4	51.5 ± 2.4	0.8 ± 1.3	1,582 ± 58.8	B
22		1.875 gms.	750	1.250 gms.	500	4	50.5 ± 2.9	4.0 ± 4.0	1,500 ± 91.7	B
23		2.625 gms.	750	....	....	4	56.5 ± 0.6	+2.3 ± 2.2	1,784 ± 49.1	B
24		2.625 gms.	750	1.750 gms.	500	4	51.0 ± 2.1	3.0 ± 3.5	1,516 ± 51.7	C
	Check (untreated)	....	....	....	....	18	54.2 ± 1.2	23.1 ± 5.0	1,308 ± 85.1	A

* Treatments 5 and 17 are equivalent in mercury content, as are also treatments 6 and 18, 7 and 19, 8 and 20, 9 and 21, 10 and 22, 11 and 23, and 12 and 24.

† Based on 3100 seeds sown in each plat.

TABLE 13. LIQUID TREATMENTS ON BEDS OF NORWAY SPRUCE SOWN AT THE KEENE NURSERY ON NOVEMBER 4, 1926

Treatment no.	Treatment		Number of replications	Results	
	Material employed	Amount per square foot applied in 500 cubic centimeters of water		Final stand per 9-row plat	Condition of seedlings at end of first growing season
1 2	Aluminum sulfate*	3.544 gms. 7.087 gms.	5 5	588±27.0 608±39.0	Normal Slightly stunted in *three* plats
3 4	Bayer compound	0.750 gm. 1.500 gms.	5 5	715±30.4 719±24.8	Normal Normal
5 6	Mercuric chloride	0.1773 gm. 0.3546 gm.	5 5	686±33.0 727±26.4	Slightly stunted in *one* plat Severely stunted in *all* plats
7 8	Semesan	0.750 gm. 1.500 gms.	5 5	653±30.4 646±45.8	Slightly stunted in *one* plat Severely stunted in *all* plats
9 10	Sulfuric acid*	3/64 fl. oz. 5/64 fl. oz.	5 5	518±36.0 527±19.7	Normal Slightly stunted in *three* plats
11 12	Uspulun	0.750 gm. 1.500 gms.	5 5	559±20.2 427±28.8	Slightly stunted in *one* plat Severely stunted in *all* plats
	Check (untreated)	....	12	587±24.5	Normal

* Due to an error in calculation, the aluminum sulfate and the sulfuric acid were used at half the strength originally planned.

It is rather surprising to note that the Bayer compound, shown by greenhouse experiments[8] to be more toxic than equal concentrations of Uspulun, was in this case non-injurious.

The results with liquid treatments on spring-sown beds of 1927 are presented in table 14. The plats of experiment B were sown on April 21, and those of experiment A on May 7, both at the rate of 3150 seeds. The two series were located several hundred yards apart.

In experiment A, all of the treatments caused a considerable reduction in the amount of damping-off and thereby increased the total emergence and final stand. The low percentage of emergence noted for all the plats is due to the fact that approximately only 25 per cent of the seed were viable. The three mercury materials gave the greatest reduction in percentage of damping-off. However, the stronger applications of these materials caused decided chemical injury. This was manifested by a failure of the seedlings to continue top growth. In normal seedlings the main axis elongated from one-half to three-fourths of an inch beyond the origin of the first leaves, while in severely stunted plants only one-fourth inch of such stem growth had occurred. It is doubtful whether the slight injury noted for the weaker mercury treatments is of any practical importance.

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[8] Unpublished results obtained by the writer.

TABLE 14. LIQUID TREATMENTS ON SPRING-SOWN BEDS OF NORWAY SPRUCE AT THE KEENE NURSERY, SEASON OF 1927

	Treatment no.	Treatment		Number of replications	Results			
		Material employed	Amount per square foot applied in 500 cubic centimeters of water		Percentage of emergence	Percentage of damping-off after emergence	Final stand per 9-row plat	Chemical injury
Experiment A	1	Aluminum sulfate	7.087 gms.	5	23.6 ± 0.7	13.2 ± 1.1	656 ± 25.3	None
	2		10.631 gms.	5	23.2 ± 0.7	9.8 ± 1.5	662 ± 29.5	None
	3		14.175 gms.	5	24.0 ± 0.6	13.4 ± 1.7	654 ± 19.2	None
	4*	Mercuric chloride	0.1773 gm.	5	23.0 ± 0.7	6.8 ± 1.1	674 ± 24.3	Slight
	5		0.3546 gm.	5	25.0 ± 1.0	7.0 ± 0.4	729 ± 28.0	Medium
	6	Semesan	0.750 gm.	5	18.2 ± 0.5	3.8 ± 1.3	547 ± 15.7	Slight
	7		1.500 gms.	5	19.0 ± 1.0	5.0 ± 0.5	570 ± 29.2	Medium to severe
	8	Sulfuric acid	3/32 fl. oz.	5	20.8 ± 0.4	17.0 ± 2.6	546 ± 27.5	None
	9		4/32 fl. oz.	5	20.8 ± 0.8	12.6 ± 2.7	577 ± 37.8	None
	10		5/32 fl. oz.	5	20.2 ± 0.5	11.2 ± 1.7	561 ± 19.4	None
	11*†	Uspulun	0.750 gm.	5	17.4 ± 0.5	7.2 ± 1.2	513 ± 16.3	Slight
	12		1.500 gms.	3	14.3 ± 0.2	6.3 ± 2.6	416 ± 13.8	Severe
		Check (untreated)	....	12	11.2 ± 0.7	58.4 ± 2.6	160 ± 15.3	....
Experiment B	1	Aluminum sulfate	7.087 gms.	3	27.3 ± 1.2	10.7 ± 1.3	779 ± 41.1	None
	2		14.175 gms.	3	29.3 ± 0.6	4.3 ± 1.1	880 ± 28.4	None
	3	Sulfuric acid	3/32 fl. oz.	3	26.7 ± 1.3	5.7 ± 0.8	785 ± 44.7	None
	4		5/32 fl. oz.	3	26.0 ± 0.4	10.0 ± 0.7	736 ± 19.7	None
		Check (untreated)	....	4	20.8 ± 2.9	36.5 ± 6.8	454 ± 9.8	....

* Treatments 4 and 11 of experiment A are equivalent in mercury content, as are also treatments 5 and 12.

† The plats of this treatment are not so nearly uniformly distributed as are those of other treatments.

Eliminating, then, those treatments which caused appreciable injury, it is of interest to note the differences that occurred in the final stand of healthy seedlings (figure 4). In general, plats treated with mercuric chloride and aluminum sulfate yielded the greatest number, and those with Uspulun the lowest, while sulfuric acid and Semesan ranged between these two.

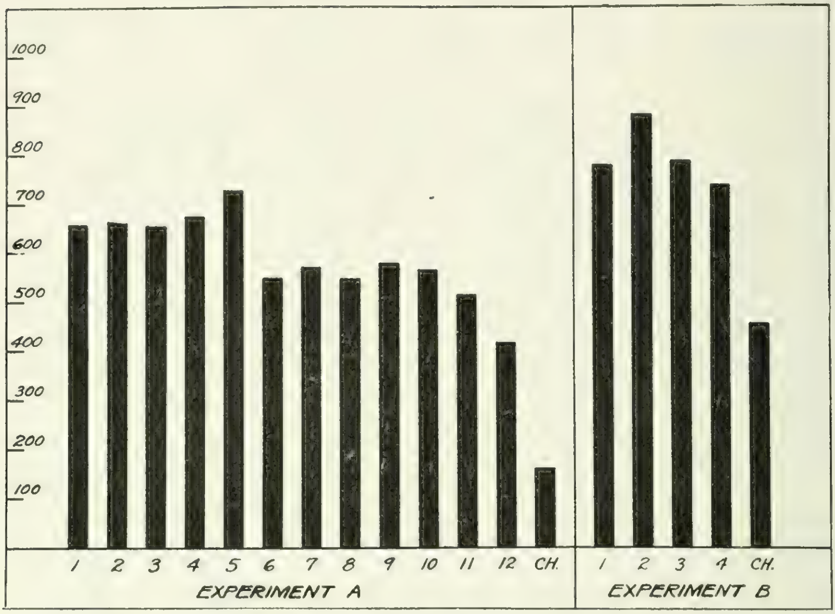

FIGURE 4. FINAL STAND OF NORWAY-SPRUCE SEEDLINGS IN PLATS TREATED WITH LIQUID APPLICATIONS OF CHEMICALS

The numbers below the columns refer to the treatment numbers given in table 14

The results of experiment B indicate that both aluminum sulfate and sulfuric acid were effective in considerably reducing the incidence of disease.

Norway spruce — Treatments with dry chemicals. Copper carbonate and several organic mercury dusts were used in seed and soil treatments on both spring- and fall-sown beds of norway spruce in 1926. The results were similar to those on red pine, which was included in the same plats. Since these tests have already been discussed under red pine, they are not considered in detail here. It will be recalled, however, that soil applications of the mercury dusts gave rather promising results on the spring-sown beds.

During the season of 1927, an extensive experiment was conducted with applications of aluminum sulfate and both organic and inorganic mercury materials, on beds of this species. The experimental plats were located adjacent to those of the liquid treatments on spruce of that season, with which they are comparable.

The high incidence of disease in the check plats (table 15) is evidenced not only by the high percentage of damping-off after emergence, but also by the low percentage of emergence. With the exception of the smaller applications of several materials, all of the treatments reduced the disease to a negligible amount (Plate VI, 1 and 2).

The larger applications of mercuric chloride and mercurous chloride caused medium to severe stunting, similar to that described for several of the liquid treatments on this species. The very slight stunting noted from the smaller applications of these materials is of no practical importance.

From the data on final stand, it is evident that the two largest applications of aluminum sulfate, the two treatments with Bayer II–6, the larger applications of Bayer dust, Dipdust, and Semesan Bel, and the smaller applications of mercuric chloride and mercurous chloride, were practically equally effective (figure 5).

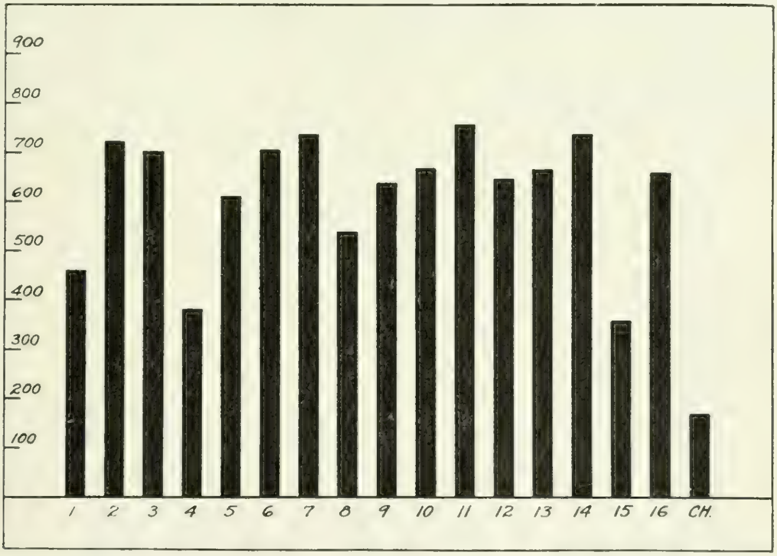

FIGURE 5. FINAL STAND OF NORWAY-SPRUCE SEEDLINGS IN PLATS TREATED WITH DRY APPLICATIONS OF CHEMICALS

The numbers below the columns refer to the treatment numbers given in table 15

TABLE 15. TREATMENTS WITH DRY CHEMICALS ON BEDS OF NORWAY SPRUCE SOWN AT THE KEENE NURSERY ON MAY 6, 1927

Treatment no.	Treatment		Number of replications	Results			
	Material employed	Amount applied per square foot		Percentage of emergence ‡	Percentage of damping-off after emergence	Final stand per 9-row plat	Chemical injury
1	Aluminum sulfate	3.544 gms.	5	18.8 ± 1.5	24.0 ± 3.5	460 ± 52.2	None
2		7.087 gms.	5	27.2 ± 1.2	14.4 ± 2.6	722 ± 22.5	None
3		10.631 gms.	5	25.2 ± 1.5	10.8 ± 2.5	701 ± 40.4	None
4	Bayer dust	2 gms.	5	18.6 ± 0.7	34.6 ± 2.8	385 ± 26.7	None
5		4 gms.	5	23.4 ± 1.2	18.4 ± 2.5	611 ± 46.3	None
6	Bayer II–6	4 gms.	5	24.2 ± 1.0	6.8 ± 1.7	705 ± 33.4	None
7		6 gms.	5	26.4 ± 1.8	11.2 ± 2.6	737 ± 51.1	None
8	Dipdust	2 gms.	5	21.0 ± 0.4	19.0 ± 2.4	539 ± 26.2	None
9		4 gms.	5	22.3 ± 1.3	9.5 ± 1.4	637 ± 41.8	None
10*	Mercuric chloride	0.1773 gm.	5	23.2 ± 1.1	8.2 ± 1.8	669 ± 33.7	Very slight
11		0.3546 gm.	4	25.0 ± 1.3	2.5 ± 2.2	757 ± 31.5	Medium to severe
12*	Mercurous chloride	0.1542 gm.	5	22.0 ± 1.2	7.0 ± 1.8	647 ± 44.7	Very slight
13		0.3084 gm.	5	21.4 ± 1.2	2.0 ± 0.8	663 ± 37.6	Medium to severe
14		0.3084 gm.†	5	23.8 ± 0.9	6.8 ± 1.3	737 ± 14.8	Medium to severe
15	Semesan Bel	2 gms.	4	16.0 ± 1.6	29.5 ± 2.3	357 ± 41.7	None
16		4 gms.	4	25.3 ± 1.4	16.8 ± 2.7	658 ± 48.1	None
	Check (untreated)	....	17	11.6 ± 0.7	57.2 ± 2.8	168 ± 19.6	

* Treatments 10 and 12 are equivalent in mercury content, as are also treatments 11, 13, and 14.
† Applied in 250 cc. water immediately after seeding.
‡ Based on 3150 seeds sown in each plat.

White pine — Liquid treatments. An experiment was conducted with liquid treatments of several mercury materials on beds of white pine at the Keene nursery in 1926. Seed were sown on May 20 at the rate of 3000 to each plat. Since only about 25 per cent of the seed were viable, the percentage of emergence was low. Field observations showed no evidence of chemical injury at the time of emergence. Severe damping-off occurred in the check plats, while practically none was apparent in the treated plats. The data on final stand presented in table 16 demonstrate that nearly all of the treatments were effective in producing from 200 to 400 more seedlings than were recorded for the check plats. The increases were for the most part easily significant.

It is interesting to note that in nine out of ten cases in which second applications were made, the final stand was lower than for the corresponding plats receiving but a single application. Field observations showed that many seedlings were killed or stunted by the second applications of mercuric chloride, while slight stunting occurred from second applications of the other materials. Observations were repeated at the end of the second growing season, and the plats were classified on the basis of the size of seedlings contained in them. In class A are included those plats on which the majority of the seedlings were of very excellent stock, in class B those on which the seedlings were of fair size, and in class C those on which the majority of the seedlings were of decidedly inferior quality and would therefore normally be discarded. The average stock used for transplanting at the Keene nursery ranges within the classes A and B, and is designated by the combination A-B. Thus from the last column of table 16 it can be seen that the smaller amounts of the materials, particularly where single applications were made, gave the most promising results.

TABLE 16. LIQUID TREATMENTS ON BEDS OF WHITE PINE SOWN AT THE KEENE NURSERY ON MAY 20, 1926

Treatment no.	Treatment					Number of replications	Results	
	Material employed	Rate of application per square foot					Final stand per 9-row plat	Classification of plats
		At seeding time		Nine weeks later				
		Weight of material	Quantity of water in which applied	Weight of material	Quantity of water in which applied			
			(Cubic centimeters)		(Cubic centimeters)			
1	Bayer compound	1.250 gms.	500	....	....	4	691 ± 31.7	A-B
2		1.250 gms.	500	0.625 gm.	250	4	613 ± 32.0	A-B
3		1.875 gms.	750	....	....	4	710 ± 28.9	A-B
4		1.875 gms.	750	1.250 gms.	500	4	604 ± 40.0	B-C
5		2.500 gms.	1,000	....	....	4	650 ± 42.2	A-B
6*	Mercuric chloride	0.4137 gm.	500	....	....	4	695 ± 29.1	A-B
7		0.4137 gm.	500	0.2068 gm.	250	4	645 ± 42.2	B-C
8		0.6206 gm.	750	....	....	4	734 ± 18.0	A-B
9		0.6206 gm.	750	0.414 gm.	500	4	629 ± 23.0	B-C
10		0.8274 gm.	1,000	....	....	4	716 ± 22.7	A-B
11	Semesan	1.250 gms.	500	....	....	4	816 ± 27.9	A-B
12		1.250 gms.	500	0.625 gm.	250	4	707 ± 50.1	B
13		1.875 gms.	750	....	....	4	710 ± 16.1	B
14		1.875 gms.	750	1.250 gms.	500	4	789 ± 24.3	B-C
15		2.500 gms.	1,000	....	....	4	761 ± 44.7	B
16	Uspulun	1.250 gms.	500	....	....	4	727 ± 41.2	A-B
17		1.250 gms.	500	0.625 gm.	250	4	668 ± 37.6	B
18*		1.750 gms.	500	....	....	4	772 ± 27.6	B
19		1.750 gms.	500	0.875 gm.	250	4	704 ± 69.9	B-C
20		1.875 gms.	750	....	....	4	824 ± 27.5	A-B
21		1.875 gms.	750	1.250 gms.	500	4	783 ± 23.1	B
22		2.625 gms.	750	....	....	4	763 ± 06.5	B
23		2.625 gms.	750	1.750 gms.	500	4	713 ± 37.4	B-C
24		2.500 gms.	1,000	....	....	4	603 ± 49.3	B
25		3.500 gms.	1,000	....	....	4	767 ± 37.7	B
	Check (untreated)	....	....	....	....	20	412 ± 31.8	A-B

* Treatments 6 and 18 are equivalent in mercury content, as are also treatments 7 and 19, 8 and 22, 9 and 23, and 10 and 25.

The results obtained with liquid treatments on this species during the season of 1927 are recorded in table 17. The plats of experiment A were sown on April 21, and those of experiment B on May 13, at the rates of 2250 and 1800 seeds, respectively. This seed was of low viability. The final stand on the aluminum-sulfate plats of experiment A show a significant increase over that of the check, while the increases with the sulfuric-acid treatments were of doubtful significance.

TABLE 17. LIQUID TREATMENTS ON BEDS OF WHITE PINE AT THE KEENE NURSERY, SEASON OF 1927

	Treatment no.	Treatment		Number of replications	Final stand per 9-row plat
		Material employed	Amount per square foot applied in 500 cubic centimeters of water		
Experiment A	1	Aluminum sulfate	10.631 gms.	3	491 ± 9.9
	2		17.718 gms.	3	452 ± 4.5
	3	Sulfuric acid	4/32 fl. oz.	3	382 ± 29.7
	4		6/32 fl. oz.	3	365 ± 36.3
		Check (untreated)	....	6	192 ± 39.4
Experiment B	1	Aluminum sulfate	10.631 gms.	5	318 ± 23.2
	2		14.175 gms.	5	365 ± 17.5
	3		17.718 gms.	5	397 ± 28.5
	4*	Mercuric chloride	0.1182 gm.	6	349 ± 25.3
	5		0.2069 gm.	6	320 ± 25.6
	6	Semesan	0.500 gm.	6	389 ± 12.9
	7		0.875 gm.	6	359 ± 24.8
	8	Sulfuric acid	4/32 fl. oz.	5	328 ± 33.3
	9		5/32 fl. oz.	5	259 ± 17.9
	10		6/32 fl. oz.	5	288 ± 12.7
	11*	Uspulun	0.500 gm.	6	403 ± 18.7
	12		0.875 gm.	6	418 ± 26.6
		Check (untreated)	....	13	267 ± 18.7

* Treatments 4 and 11 of experiment B are equivalent in mercury content, as are also treatments 5 and 12.

Unfortunately the results obtained from experiment B were somewhat vitiated by severe bird injury. As a consequence of this and of the low incidence of disease throughout the beds, the figures for final stand are significantly greater than those for the untreated plats, except for treatments 6, 11, and 12. All but treatment 9, however, gave greater absolute yields than those of the check. No chemical injury could be detected in any of the plats.

Seedlings in another experiment, in which 1 gram and 1.75 grams of Semesan and Uspulun, and 0.236 and 0.414 gram of mercuric chloride,

Plate VI

FINAL STAND OF NORWAY-SPRUCE SEEDLINGS IN BEDS TREATED WITH DRY CHEMICALS FOR CONTROL OF DAMPING-OFF

1: Plat 27, untreated; plat 25, 1/8 ounce of aluminum sulfate per square foot; plat 26, 2/8 ounce of aluminum sulfate per square foot; plat 28, 3/8 ounce of aluminum sulfate per square foot

2: Plat 33, untreated; plat 31, 4 grams of Bayer II–6 per square foot; plat 32, 6 grams of Bayer II–6 per square foot; plat 34, 2 grams of Dipdust per square foot

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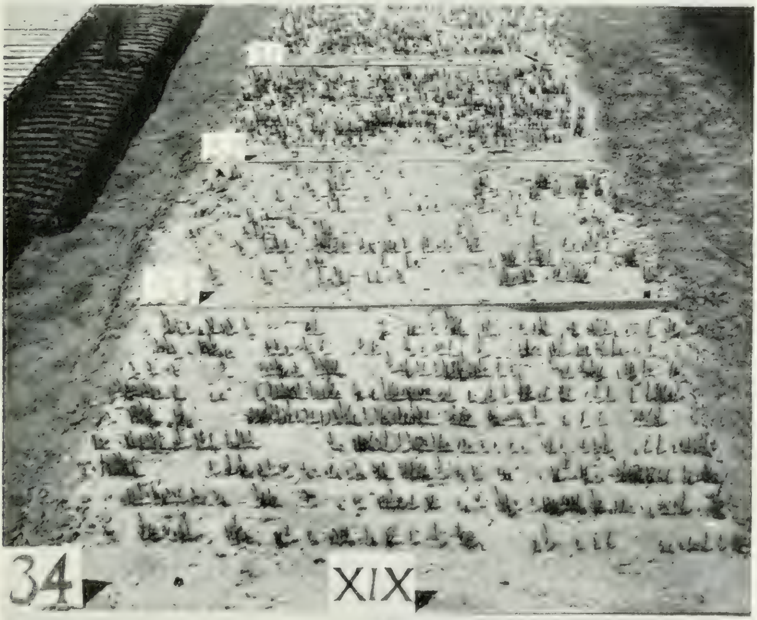

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were applied per square foot, respectively, were practically ruined through bird injury. Examination of the seedlings showed no evidence of stunting, even with the larger applications.

White pine — Treatments with dry chemicals. White pine was used, along with red pine and norway spruce, in the preliminary experiments with seed and soil applications of copper carbonate and several organic mercury dusts during the spring of 1926. The results obtained were less favorable than those with the other two species, there being lower absolute yields in some of the treated plats than in the checks.

During the spring of 1927 an extensive series of tests was conducted with applications of aluminum sulfate and a number of mercury materials. Seed of very low viability was sown on May 12, at the rate of 1800 per plat. The results of the experiment are recorded in table 18. Here

TABLE 18. TREATMENTS WITH DRY CHEMICALS ON BEDS OF WHITE PINE SOWN AT THE KEENE NURSERY ON MAY 12, 1927

Treatment no.	Treatment		Number of replications	Final stand per 9-row plat
	Material employed	Amount applied per square foot		
1	Aluminum sulfate	7.087 gms.	5	238 ± 16.5
2		10.631 gms.	5	280 ± 23.2
3		14.175 gms.	5	242 ± 21.4
4	Bayer dust	2 gms.	5	218 ± 25.0
5		4 gms.	5	275 ± 25.9
6		6 gms.	5	285 ± 9.2
7	Bayer II–6	2 gms.	5	289 ± 17.5
8		4 gms.	5	235 ± 10.3
9		6 gms.	5	274 ± 16.7
10	Dipdust	2 gms.	5	257 ± 15.8
11		4 gms.	5	248 ± 17.4
12		6 gms.	5	248 ± 10.4
13*	Mercuric chloride	0.2364 gm.	5	240 ± 15.1
14		0.4137 gm.	5	230 ± 10.0
15*	Mercurous chloride	0.2056 gm.	5	225 ± 18.2
16		0.3598 gm.	5	247 ± 16.4
17		0.3598 gm.†	5	275 ± 21.0
18	Semesan Bel	2 gms.	5	289 ± 16.0
19		4 gms.	5	265 ± 25.2
20		6 gms.	5	244 ± 5.6
21	Semesan Jr.	2 gms.	5	206 ± 18.8
22		4 gms.	5	253 ± 11.3
23		6 gms.	5	277 ± 15.0
	Check (untreated)	....	24	153 ± 12.7

* Treatments 13 and 15 are equivalent in mercury content, as are also treatments 14, 16, and 17.

† Applied in 250 cc. water immediately after seeding.

again bird injury was so severe that detailed comparisons between different treatments cannot be made. All treatments except those numbered 4, 15, and 21, however, gave significant increases in final stand over that of the check. No evidence of stunting was apparent from any of the treatments.

Miscellaneous species. During the season of 1927, tests were made with liquid treatments of mercuric chloride, sulfuric acid, and Uspulun on beds of austrian and scotch pine, colorado, engelmann, and white spruce, and european larch. Four rows each of two different species were sown in each plat, and three replications of each treatment were made. Since the incidence of disease was low in most of the beds, the experiment was of value chiefly in determining the effects of the treatments upon the growth of seedlings during the first season. It is apparent from table 19 that for the most part the treatments were not injurious to the two pine species. The larger applications of mercuric chloride and Uspulun caused injury to colorado spruce, while only the acid treatments were satisfactory on engelmann spruce. European larch and white spruce were severely stunted by all the mercury treatments, while the two concentrations of sulfuric acid caused slight and medium stunting, respectively.

TABLE 19. EFFECT OF LIQUID TREATMENTS UPON AUSTRIAN AND SCOTCH PINE, COLORADO, ENGELMANN, AND WHITE SPRUCE, AND EUROPEAN LARCH

Treatment			Chemical injury					
Treatment no.	Material employed	Amount applied per square foot in 500 cubic centimeters of water	On austrian pine	On scotch pine	On colorado spruce	On engelmann spruce	On white spruce	On european larch
1*	Mercuric chloride	0.2364 gm.	None	Slight	None	Slight to medium	Severe	Severe
2	Mercuric chloride	0.3546 gm.	None	Slight	Slight to medium	Medium to severe	Severe	Very severe
3	Sulfuric acid	3/32 fl. oz.	None	None	None	None	Slight	Slight
4	Sulfuric acid	5/32 fl. oz.	None	None	None	Very slight	Medium	Medium
5*	Uspulun	1 gm.	None	None	None	Slight to medium	Severe	Severe
6	Uspulun	1.5 gms.	None	None	Slight to medium	Medium to severe	Severe	Very severe

* Treatments 1 and 5 are equivalent in mercury content, as are also treatments 2 and 6.

Effect of the treatments on germination and subsequent growth of the seedlings

In presenting the results of the soil-treatment experiments, attention was called to the injurious effects noted from the application of chemicals in certain cases. Such injury, when it resulted from treatments made at the time of seeding, was manifested by a retardation of root development and a failure of the plant to continue normal elongation of the stem and production of leaves. Only with the copper-carbonate treatments was there evidence of that extreme type of injury expressed by an almost entire prevention of root development and by the failure of the cotyledons to grow out of the seed coats. Injury sufficient to produce killing of dormant seed was not encountered in any of these experiments. When injury resulted from a single treatment made at the time of seeding, the rate of application was in most cases higher than that necessary for effective control of the disease.

With the applications made after the seedlings had emerged, chemical injury frequently resulted, in some cases even from treatments that were ineffective in preventing the spread of the disease. This injury was manifested by both checking of root development and direct killing of stems and cotyledons.

A marked variation was found among the different species of conifers in susceptibility to chemical injury. Norway spruce was much more susceptible to such injury than were red pine and white pine. Limited tests with several other species indicated that austrian pine and scotch pine reacted similarly to red pine and white pine. Colorado spruce and engelmann spruce, however, like norway spruce, were more liable to such injury. White spruce and european larch were even more susceptible to chemical injury than were the three species of spruce noted above.

Weed control

Many investigators have reported a reduction in the number of weeds occurring in coniferous seedbeds where chemicals were applied for the control of damping-off. Similar results were noted throughout these investigations, the weeds being much fewer in most of the treated plats. The information obtained was largely observational in character, since in most of the plats weeding operations were carried out as a part of the regular nursery practice. In one experiment with colorado and engelmann spruce, however, the weeds were not removed. The condition existing in a few of the plats three months after seeding is illustrated in figure 6.

There were some indications that aluminum sulfate and sulfuric acid were in general more effective in weed control than the mercury materials, and that liquid treatments were more effective than the applications of dry chemicals.

Relative cost of different treatments

A consideration of soil treatments for controlling damping-off of conifers necessarily involves the question of the expense of making such treatments.

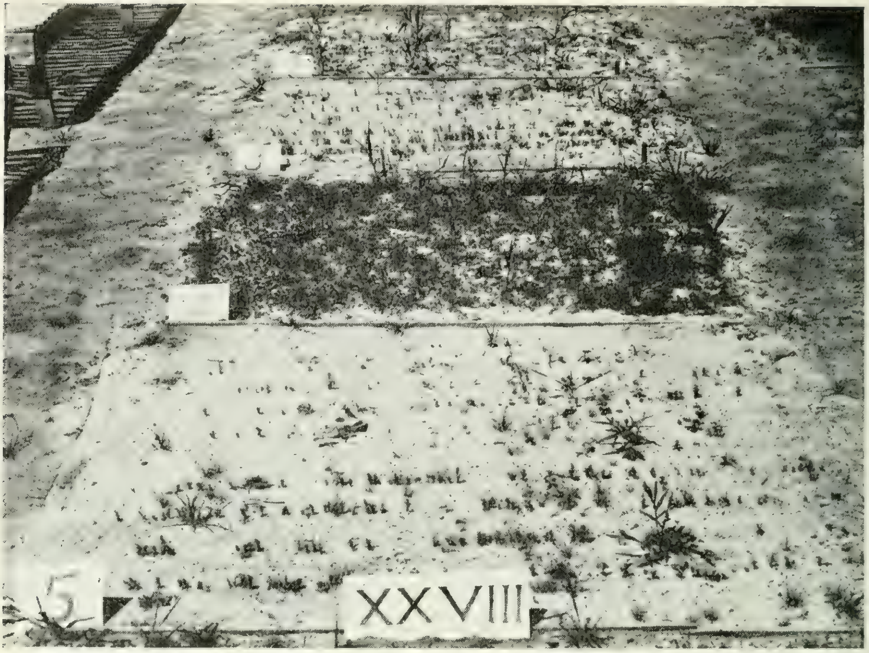

FIGURE 6. WEED CONTENT IN PLATS OF COLORADO AND ENGELMANN SPRUCE

The poor stand is due to low viability of the seed. The abundant spreading weed in the check plat (4) is chickweed (*Stellaria media* [L.] Cyrill.). Plat 2 was treated with 0.3546 gram of mercuric chloride, and plats 3 and 5 with 3/32 fluid ounce and 5/32 fluid ounce respectively, of sulfuric acid, per square foot

In table 20 are given the costs of representative materials which were

TABLE 20. RELATIVE COST AND RATE OF APPLICATION FOR DIFFERENT MATERIALS USED IN SOIL-TREATMENT EXPERIMENTS

Material	Type of application	Rate of application per square foot	Approximate rate of application per 1000 square feet	Approximate cost of material per 1000 square feet†
Aluminum sulfate.	Dry.....	10.631 to 14.175 gms....	24 lbs. 7 oz. to 31 lbs. 4 oz..	$0.39 to $0.52
Aluminum sulfate.	Solution..	10.631 to 14.175 gms....	24 lbs. 7 oz. to 31 lbs. 4 oz..	$0.39 to $0.52
Bayer II–6.......	Dry.....	4 gms..................	8 lbs. 13 oz...............	$14.11
Dipdust	Dry.....	4 gms..................	8 lbs. 13 oz...............	$14.11
Semesan Bel......	Dry	4 gms..................	8 lbs. 13 oz...............	$14.11
Mercuric chloride..	Dry.....	0.1182 to 0.1773 gm.*...	4.2 oz. to 6.3 oz..........	$0.59 to $0.88
Mercuric chloride..	Solution..	0.1182 to 0.1773 gm.*...	4.2 oz. to 6.3 oz..........	$0.59 to $0.88
Mercurous chloride	Dry.....	0.1028 to 0.1542 gm.*...	3.6 oz. to 5.4 oz..........	$0.63 to $0.94
Semesan.........	Solution..	0.50 to 0.75 gm.........	1 lb. 2 oz. to 1 lb. 10 oz....	$2.87 to $4.30
Sulfuric acid......	Solution..	4/32 to 5/32 fl. oz.......	15 lbs. to 18 lbs. 12 oz..... (3.9 qts.) (4.9 qts.)	$1.05 to $1.31
Uspulun.........	Solution..	0.50 to 0.75 gm.*.......	1 lb. 2 oz. to 1 lb. 10 oz....	$2.87 to $4.30

* The applications of mercuric chloride, mercurous chloride, and Uspulun noted here are equivalent in mercury content.

† Based on current prices of the grades used at the Keene nursery, shipping costs not included.

found effective in these experiments. Of the different materials tested, aluminum sulfate was the most inexpensive. The cost given is for the commercial grade, which was equally as effective as the more expensive technical grade. The organic-mercury preparations, particularly the dusts, were the most expensive of the materials used. In view of the fact that most of these have but recently appeared on the market, it is possible that the prices will be reduced with greater volume of production.

The factors of time and labor involved in applying treatments must also be considered along with the cost of chemicals. From this point of view the application of dry chemicals has much in its favor, since the inconvenience and the expense of handling large quantities of water are avoided. A further advantage of the use of dry chemicals is the ease with which the materials can be worked into the soil during the regular operations of preparing the seedbeds. The only added labor involved is that required to sift the chemical on the surface of the beds prior to the final raking.

GENERAL DISCUSSION AND CONCLUSIONS

A number of widely varying factors are necessarily involved in any discussion of damping-off. It is perhaps fortunate that in most of these investigations the causal organism was fairly constant throughout. It has been pointed out that while a number of different forms of Rhizoctonia were isolated from diseased seedlings, the majority of strains from the Keene nursery were strikingly similar. This fact is of advantage in making different soil-treatment experiments comparable to one another. One cannot, of course, draw too definite conclusions as to the results which may be expected where fungi other than Rhizoctonia are involved. Some information is available, however, from the Ithaca experiment, where Pythium was present throughout the beds and caused at least a part of the losses.

During the course of the early field experiments, considerable attention was given to the most desirable time for making soil treatments. It was hoped that some of the mercury materials would be found effective for use in checking epiphytotics of damping-off if applied after the disease first became evident. It was soon realized, however, that losses resulting from the killing of the germinating seeds and seedlings by the fungus before they appeared above ground, made such treatments impracticable.

One of the most outstanding features of these investigations has been the rather uniformly successful control of damping-off obtained through a single application at the time of seeding, with certain concentrations of nearly all of the materials tested. This is particularly striking in view of the heavy losses in the untreated plats. Thus, in many experiments the increase in final stand with most treatments over that of the check was as high as 200 to 300 per cent.

The experiments of 1927 have demonstrated that successful control of damping-off can be obtained by the use of dry applications of aluminum sulfate, the two chlorides of mercury, and several preparations containing organic mercury compounds. As far as the writer knows, this is the first report of controlling the damping-off of conifers by this type of application. The results suggest considerable promise for the method, in point of both convenience and cost of treatments.

In using the dry chemicals at nurseries where the broadcast method of sowing is practiced, it will be necessary to disinfect the thin layer of sand which is used for covering the seed. This can be done by mixing the chemical with the sand prior to covering, or by adding a very small amount of the chemical to the surface of the beds after the seed has been covered.

Another problem arises with the use of dry chemicals, in that, with the exception of the organic-mercury preparations, none of the materials used in these tests can now be obtained on the market in the most desirable form. In the case of aluminum sulfate there is available a pulverized form from which can be sifted out a product sufficiently finely divided to be applicable for this type of treatment. It would be highly desirable, however, to have available a much more finely divided preparation of aluminum sulfate. The problem is being taken up with the manufacturers of this material.

There is also the problem of devising a suitable apparatus for applying the dry chemicals. The rather crude methods used in the present tests were effective, but they could be improved upon by employing some type of sifter or other device for obtaining a more rapid and uniform distribution of the fungicide.

With regard to the relative merits of the different chemicals, the results of these investigations indicate that liquid treatments with aluminum sulfate,[9] mercuric chloride, Semesan, Uspulun, or sulfuric acid, and with dry treatments of aluminum sulfate, Bayer II–6, or either of the two chlorides of mercury, were practically equally effective in controlling damping-off. Thus, in certain experiments significant differences could not be demonstrated between the results with these materials. In other experiments such differences existed, but the relative position of a given chemical was not constant throughout several experiments. Bayer dust, which was used only in light applications, gave less favorable results than did the above-named materials. However, since Bayer dust contains the same mercury compound (except in smaller amounts) as does Bayer II–6, it is believed that heavier applications would probably be as effective as the latter preparation. Dipdust was not uniformly so effective as the other materials. No explanation is offered, since the content of organic mercury was higher than that of other preparations which gave more favorable results. Semesan Bel, which was used to a limited extent, was effective in the larger applications. Semesan Jr. gave excellent results in several tests. The results with the two last-named materials suggest that equal amounts of chlorophenol mercury are more effective when used in solution than when applied dry.

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[9] Suggested rates of application with aluminum sulfate were furnished to the writer by Dr. Carl Hartley, of the Office of Forest Pathology, United States Department of Agriculture.

An adequate comparison of the mercury compounds on the basis of mercury content has not been made in these experiments. The data at hand, however, are suggestive in this connection. The results of the liquid treatments have indicated that mercury-equivalent solutions of mercuric chloride and chlorophenol mercury are approximately equally effective. In the dry form the two chlorides of mercury gave similar results when applications containing equal amounts of mercury were used. Nitrophenol mercury was more effective than chlorophenol mercury of equal mercury content. The results with dry applications suggest further that both compounds of inorganic mercury were more toxic to seedlings, and gave greater reductions in damping-off, than did either of the organic compounds, where the comparison is made on the basis of mercury content.

The evidence from the soil-treatment experiments definitely indicates that the materials here used exerted not only a disinfecting action at the time of their application, but a protective action as well during the period of emergence and for some time thereafter. In no instances were precautions taken to prevent reinfection of treated beds. The nursery practices made reinfection from paths easily possible, yet damping-off did not appear at the margins of the beds more frequently than elsewhere. The evidence of chemical injury from certain concentrations also indicates that the materials were present in active form some weeks after treatments were made. This is well illustrated by the fall applications on norway spruce (table 13), where injury was noted on seedlings emerging during early spring, five months after the treatments were made. Whether the chemicals remained in an active state throughout the intervening period, or whether they were brought into active form during the germination processes, is not known.

SUMMARY

This paper brings together the results of three years of investigations on the damping-off of conifers, conducted at Ithaca, New York, and at Keene, New Hampshire.

Isolations from diseased seedlings at the Ithaca nursery indicated that Rhizoctonia and Pythium were the pathogenes causing the disease. At the Keene nursery, Rhizoctonia was practically the only organism involved. Pure cultures were obtained in more than 900 cases from approximately 1300 isolations. Isolations from six other nurseries yielded a high percentage of Rhizoctonia and Fusarium, and in a few cases Alternaria.

Rhizoctonia and Pythium were highly virulent in greenhouse inoculation tests. The few tests with Fusarium and Alternaria gave negative results.

Cross-inoculation experiments with thirteen strains of Rhizoctonia were made on six species of vegetables and five species of conifers. The results indicated considerable differences in virulence between the strains. In only a few cases did these differences seem related to the species of suscept from which the strains were isolated. Cultural characters of twenty-three strains of Rhizoctonia (including the ones noted above) are described on four artificial media. No correlation could be made between the results of the cross-inoculation studies and those of the cultural studies. With one possible exception the strains used in these tests were considered to fall within the limits of *Rhizoctonia solani* Kühn.

A limited number of observations failed to show any definite relation between damping-off and either fall seeding or application of muck to the seedbed.

The application of chemicals after emergence of the seedlings was found not to be a practicable means of controlling the disease.

Single applications of chemicals at the time of seeding, however, with any one of a number of different materials, were found to give excellent control of damping-off under conditions where extremely high losses occurred in the untreated plats. In determining the optimum rate of application, injury was noted in certain cases in which the amount of chemical used was greater than that necessary to control the disease. Austrian, red, scotch, and white pine were found to be least susceptible to such injury. Colorado, engelmann, and norway spruce were more liable to injury than the four species of pine. White spruce and european larch were severely stunted by chemical treatments in one experiment.

Applications of dry chemicals at the time of preparing the seedbeds were found to be as effective as liquid treatments.

On the basis of these experiments, it is concluded that a single treatment at the time of seeding, with liquid applications of aluminum sulfate, mercuric chloride, Semesan, sulfuric acid, or Uspulun, or with dry applications of aluminum sulfate, Bayer II–6, mercuric chloride, or mercurous chloride, are equally effective in controlling damping-off of norway spruce, red pine, and white pine. Bayer dust and Dipdust, in the amounts used, gave less satisfactory results than did the treatments noted above. Semesan Bel and Semesan Jr. were effective in the limited tests made with them. A suggested rate of application, together with the cost of materials, is presented. Aluminum sulfate is less expensive than any of the other materials. The organic mercury preparations, particularly the dusts, are the most expensive of the materials tested.

The results of these experiments indicate that at least some of the chemicals exerted not only a disinfecting action, but also a protective action for a number of weeks after the application.

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THE BACTERIAL DISEASES OF THE BEAN

A COMPARATIVE STUDY

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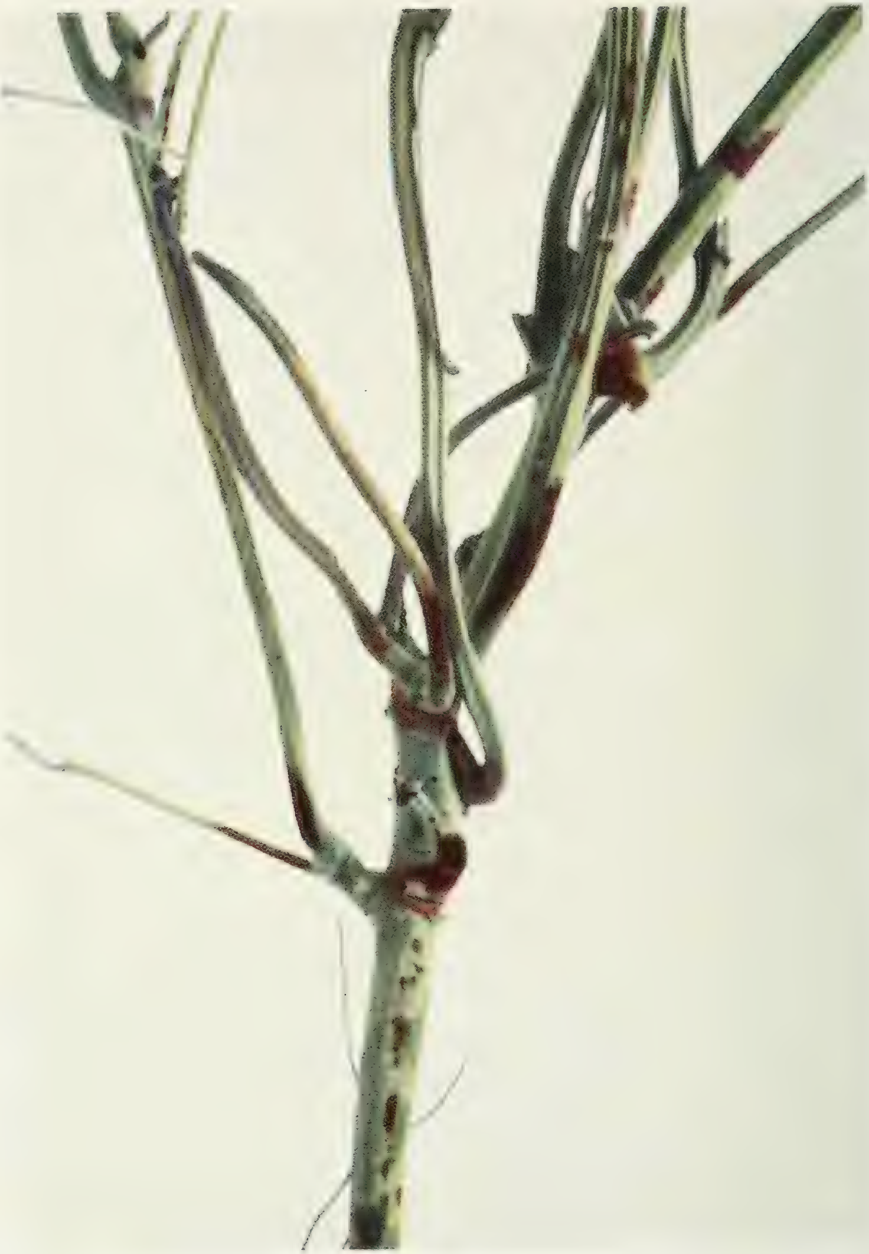

DISEASED STEMS AND PETIOLES OF A RED KIDNEY BEAN PLANT

These lesions are caused by *Phytomonas phaseoli*

THE BACTERIAL DISEASES OF THE BEAN

A COMPARATIVE STUDY

Walter H. Burkholder

The purpose of the investigation here reported was to separate and to distinguish, among the various diseases of the bean (*Phaseolus vulgaris* L.), those that are of bacterial origin. A number of such diseases occur on this crop, with symptoms for the most part similar and in some cases apparently indistinguishable from one another. The questions naturally arise as to how many of these diseases there are, which ones are causing economic losses to the crop, and how they may be recognized one from another. A knowledge of these phases of the subject is essential for the investigator before further work can be done effectively on any one of the diseases, since he must be able to recognize the disease with which he is working and to eliminate or be aware of the remaining bacterial diseases in his field of experimentation.

The bacterial disease of the bean caused by *Phytomonas phaseoli* has been recognized for a considerable period of time, but the diseases caused by *Phyt. flaccumfaciens* and *Phyt. medicaginis* var. *phaseolicola* have been known only during the past four or five years. Since these last-named diseases have been described, no comparative study has been made of the three; and, because they are so similar in many respects, confusion has arisen as to their distinctiveness and as to their identity. It was hoped in undertaking this investigation to clear up some of this confusion. In the progress of the work, however, it soon was found that still other bacterial diseases of this crop exist which further complicate the problem. Six distinct diseases have been recognized in this investigation, and in certain of these the pathogene appears to be composed of several different races. Consequently the entire problem of the bacterial diseases of the bean is more complicated than formerly it was thought to be.

In separating the various diseases and distinguishing them one from another, the work naturally falls into three parts: first, the symptomatology of the diseases on the bean; secondly, the host range of the pathogenes; and thirdly, the etiology, a study of the morphology and the cultural characteristics of the pathogenes. Other phases of the problem are numerous and important, but they are not phases that would facilitate the separation of these diseases which are under consideration. In presenting each disease separately in this memoir, the above three divisions of the problem have been dealt with mainly. What is known concerning the life history of the organism, however, has not been neglected. In treating this problem in such a manner it has seemed justifiable to duplicate certain of the previous work done with these diseases. All cultural characteristics of the pathogenes have been determined under identical conditions (and on the same media), which is an essential precaution in showing similarities and distinct differences. The symptomatology of the various diseases on the bean host has been studied under similar conditions, and the host range for each pathogene determined in a like manner. By following such a procedure it is felt that a certain amount of error has been eliminated which might exist in separate investigations conducted in separate laboratories.

So far as is known, only one important bacterial pathogene of the common bean has been neglected. *Phyt. phaseoli* var. *sojense*, while found primarily on the soybean, does produce an infection on the common, or garden, bean, and it is regretted that this organism was not included in the comparative study. Wolf (1924) and Miss Hedges (1924) have reported their investigations on the soybean organism, however, and find that culturally it agrees with *Phyt. phaseoli*, except for a few minor exceptions. The problem appears to be primarily one of pathogenicity. Several other bacterial pathogenes have been reported as being pathogenic to the bean, but they are ones which are of little importance to this host. *Phyt. solanaceara*, *Phyt. tobaca*, and *Phyt. aptata* might be mentioned in this class.

METHODS

In any comparative study, the methods used for conducting the experiments are of primary importance. In the following paragraphs are set forth the methods employed in making inoculation experiments and in determining or describing the pathogene in culture. Wherever a deviation from these methods is made, it is mentioned in the text.

INOCULATION EXPERIMENTS

For conducting inoculation experiments, plants were grown in a greenhouse at a relatively high temperature. The common bean (*Phaseolus vulgaris*) and many of its related species thrive best in a warm atmosphere, a vigorous succulent growth is obtained, and furthermore the plants are not so subject to damping-off as they are at a lower temperature. The clovers and the garden peas are an exception to this rule, and these plants were grown in a cooler house. The greenhouse used for the inoculation experiments on beans had a constant-temperature-control apparatus which held the house at approximately 78° to 80° F., except during bright, sunny days when the temperature would rise to 90° F., or even more, after the steam had been shut off. Under these temperature conditions, infection developed much more rapidly when plants were inoculated with such organisms as *Phytomonas phaseoli* and *Phyt. flaccumfaciens* than at lower temperatures, such as 60° to 65° F. With some of the other pathogenes, as good results were obtained at these lower temperatures but, from observation, infection was in no respect better. *Phyt. medicaginis* var. *phaseolicola* appears to spread in the field more rapidly and do more damage in a cool season than in a warm season. In the greenhouse, however, excellent infection is obtained at 80° F.

What part humidity plays is still in doubt. When inoculations were made by injuring the plants, no extra care was taken to place them in a moist atmosphere. Such inoculated plants were allowed to remain on the greenhouse benches under the usual conditions, and inoculations were made at any time during the day. When plants were inoculated by spraying with a water suspension of the organism, without injuring them in any manner, it was considered wise to place them in a moist chamber for about twenty-four hours. This procedure, however, was not based on any experimental evidence.

The technic of inoculation by injuring the plants was as follows: The pathogene was grown on an agar slant at 27° C., and its age when inoculations were made was, as a rule, from twenty-four to forty-eight hours. Seedlings were used which had advanced beyond the primary leaf stage but were still tender and succulent. A small part of the bacterial mass was placed on a very sharp pointed scalpel and the plant was stabbed with this contaminated instrument. The injury was made in most cases at the primary leaf node and extended into the pith. When pods were used in the experiments they were inoculated in a similar manner, the injury being made either on the suture or on the side.

When experiments were conducted without injuring, the inoculations were made by spraying the plants with a water suspension of the pathogene from the agar slants. The bacteria were washed from the agar with tap water and sprayed from an atomizer. The plants were then placed in a moist chamber for approximately twenty-four hours. When pods were used, the water suspension not infrequently was painted on with a camel's-hair brush.

Some confusion exists in the literature as to the correct scientific name to be applied to certain of the leguminous plants referred to in this bulletin. Bailey's *Manual of Cultivated Plants* has been followed here in so far as specific names are concerned. Where varieties have been established on the character of determinate or undeterminate growth habit, they have been disregarded.

PURE-CULTURE STUDIES

Before the cultural studies were begun, all strains of the various species and varieties of bacteria used were inoculated into bean plants. After infection had taken place and the disease had progressed to a considerable extent, the organism was reisolated from diseased tissue as far removed from the point of inoculation as possible. Dilution plates were then made and a single colony was transferred to a tube. When the pathogenicity of this culture had been proved, the cultural work was begun. It should be stated here that prior to the foregoing procedure an attempt was made to rejuvenate all strains of pathogenes which had been in pure culture for a year or more. This was done by growing them for several months in their natural habitat, that is, as pathogenes on the bean plant. It is felt by the writer that in comparative studies, especially with fungi, many of the so-called physiologic species are brought about merely by the various lengths of time during which the organism was grown in pure culture. The writer has shown that this is the case with the fungus *Fusarium martii* f. sp. *phaseoli*, and that a return to the natural habitat will cause a return to the original morphologic and physiologic state (Burkholder, 1924). It was hoped that this would hold true also with the bacterial pathogenes.

In the preparation of media, stains, and other materials, and for their method of use, the *Manual of Methods for Pure Culture Study of Bacteria*, issued by the Society of American Bacteriologists, was followed unless otherwise stated. The formulae for preparing Uschinsky's solution, Fermi's solution, and Cohn's solution, were obtained from Smith's *Bacteria in Relation to Plant Diseases*. Andrade's indicator was used in all the sugar broths. To determine cellulose digestion, a broth containing 0.2 per cent of peptone and 0.1 per cent of K_2HPO_4 was used, in which was placed a narrow strip of filter paper extending above the medium. In determining the color of pigment production of an organism in or on any of the media, Ridgway's *Color Standards and Color Nomenclature* was employed.

Variations occurred in the same strain in different lots of media made according to the same formula but with ingredients from different sources. While this was true to a certain extent with beef-extract agar, it was very evident at times with gelatin. The most striking cases are recorded later in this memoir.

In studying the bacteria, all cultures, with the exception of gelatin, were kept in an incubator at a constant temperature of 27° C. Gelatin cultures were incubated at 21° C. The 27°-C. temperature was decided on through the observation that all cultures thrived well at that temperature. Some of the organisms, however, appeared to grow equally well at a somewhat lower temperature.

In measuring individual cells the Congo red negative stain was used as described by Benians (1916). It was felt that for the careful study of morphology, and especially of size, results obtained by the direct staining methods are less nearly accurate.

THE BACTERIAL BLIGHT OF THE BEAN CAUSED BY PHYTOMONAS PHASEOLI

Although a bacterial disease of beans was reported as early as 1892 both by Beach (1893) and by Halsted (1893), Smith (1898) was the first to isolate a bacterium from the bean, prove its pathogenicity, and give the organism a name. First described as *Bacillus phaseoli*, references to the pathogene may be found in literature under the following generic names in accordance with the classification which the various writers prefer: Pseudomonas (Migula), Bacterium (Lehman and Neuman, and E. F. Smith), and Phytomonas (Bergey). In this memoir the Bergey classification is adopted.

For approximately twenty-five years after the discovery of *Phytomonas phaseoli*, all references to a bacterial disease of beans assumed this organism to be the etiological agent. Possibly in the great majority of the cases this assumption was true, and the other bacteria pathogenic to this host are, no doubt, of recent appearance. One reason for believing this, is the fact that only in recent years in the United States have the bacterial blights been so severe. It seems inconceivable that *Phyt. flaccumfaciens* or *Phyt. medicaginis* var. *phaseolicola,* two organisms which are much more destructive at times than *Phyt. phaseoli,* would have been overlooked if they were present on the bean, or that their virulence should have increased in later years. Furthermore, these various pathogenes are not limited to *Phaseolus vulgaris,* and it is entirely possible that they appeared originally on other species and that their appearance on the bean is of but recent occurrence. Pathogenic on a wild species or slightly pathogenic on some cultivated host, they very easily could have been overlooked. After they once got a foothold on the bean plant, and especially the seed stock, they spread rapidly over the country.

Since *Phyt. phaseoli* is the oldest and the best-known species of the bacteria attacking the bean, it is the one with which all the others are compared. For this reason this pathogene and the disease which it produces are treated first.

LIFE HISTORY

In order to recognize and understand certain symptoms of the disease caused by *Phytomonas phaseoli* and to differentiate them from the symptoms of the other bacterial diseases of the bean, a knowledge of certain phases of the life history of the pathogene is helpful. The term *life history* is used here in a broad sense, to designate not only the methods of overwintering of the organism, and the methods of infection, but also the tissue of the plant that is attacked and the peculiar invasive ability of the organism in the host. While these features are stressed in the following sections, what is known of the entire life history of the pathogene is also reported.

One of the most important stages in the life history of an organism, from the standpoint of primary infection and of control, is the stage in which it passes the winter. The usual way in which *Phyt. phaseoli* survives the cold months is in a dry condition in or on the bean seed. Here it is in a highly resistant state, and under laboratory conditions at Ithaca, New York, it has been known to remain viable for a period of three years. Rapp (1919), in Oklahoma, is of the opinion that the pathogene in this state will die within a year's time, and Sackett (1926), in Colorado, reports success in reducing the amount of disease by planting two-years-old seed. Temperature, moisture, and the manner of storing the seed, no doubt play an important part in such viability tests.

Muncie (1917 b) and recently Patel (1929) have shown that *Phyt. phaseoli* can overwinter also in the soil. One experiment has been conducted by the writer on this phase of the subject, and, while not conclusive, is reported here merely for what it is worth. This experiment differs from those of Muncie and Patel in that it was conducted in the field, and, although having a more practical bearing, it lacked the niceties of the laboratory experiments. A plot 100 feet by 50 feet in size was planted to beans, and, when germination had taken place and the seedling stage had passed, the plants were inoculated heavily with the pathogene. All plants became severely infected, and in the fall were not pulled and removed as is usually the case in the sections growing dry shell beans. Thus the soil was much more severely contaminated with the organism than is usual. *Phyt. flaccumfaciens* probably was present, besides *Phyt. phaseoli.* In the following spring the plot was plowed and again sown to beans. On either side, plots of similar size were used as checks. In August, when counts were made of the number of blighted plants in each plot, it was found that the checks had slightly more diseased individuals than the treated plots had. Continuous observations before this time had shown that the disease had not originated in the treated plot and spread to the checks, but had appeared simultaneously over the entire planted area. The disease had come in on the seed, or had been brought in, in some other manner, from the outside. This is merely a single experiment, and such conditions as the amount of organic material in the soil, hydrogen-ion concentration, and other related factors, might alter the case.

The fact that the bacteria in the soil can survive a winter under New York conditions is probably of no great importance. Rotation of crops is practiced, and even in the western counties, where beans are grown as a major crop, the rotation is seldom less than three years. Can the organism survive for a period of that length? From observation, probably not, since the disease is of no economic importance when seed from disease-free fields is used for planting.

Under New York conditions, infected seed introduce the pathogene into a field in approximately 100 per cent of the cases. The life history of the pathogene in relation to its host plant, then, may proceed in the following manner: With the germination of the planted seed, the bacteria become active, and their number in the cotyledons determines the severity of early infection, weather conditions being favorable. With severely infected seed the growing tip may be destroyed by the time the plant is above ground, giving rise to the so-called *snakeheads* and frequently causing the death of the seedling. This consequently reduces the chances of the particular pathogene's continuing and infecting other host plants. In less severely infected seed, or when weather conditions are less favorable for the bacteria, they may be confined at first entirely within the cotyledons. Here they may multiply in the fleshy tissue, and be washed or splashed by rain to various parts of the young growing plant. An injury is not necessary for the bacteria to cause infection, and almost any tissue above ground may be invaded. In some cases, during the early stages of development the pathogene in the cotyledons may enter the stem of the plant through the vascular system and establish itself in the xylem vessels. The walls of these vessels apparently are very resistant to the organism, since the bacteria may exist there for weeks without breaking through into other tissue. In the xylem vessels, however, the bacteria may travel throughout the plant. Their progress usually is upward, probably owing to the water stream; but they may also travel downward, and in rare cases have been recovered from the roots of diseased plants. When they are confined to the stem alone, little effect may be produced on the plant aside from a slight stunting; but when they pass into the leaves, the young growing tips, and other plant parts, wilts and necrotic lesions may result. In their passing through the xylem vessels of the pedicel into fairly mature pods and infecting the seed, no external evidence of the presence of the bacteria may be noticed. Zaumeyer (1929) has recently described in detail the tissues involved in this latter process.

The tendency for *Phyt. phaseoli* to systemically invade a plant was first noted by Barlow (1904) and was later emphasized by the writer (Burkholder, 1921). Plants so infected are not the rule in the fields of New York; nevertheless they do serve a purpose in the life history of the organism in this climate. The first month-and-a-half of the growing period of the bean plant is unfavorable for the development of the blight. June and early July are inclined to be cool, and *Phyt. phaseoli* prefers a warm temperature. In late July and during August, when the pathogene has come to the surface in systemically infected plants through various lesions, these plants act as a source of inoculum for the spread of the disease. At times, in a bean field under close observation, one may watch the spread of the blight in an ever-growing radius about such a plant. The overwhelming majority of the leaf and pod lesions which one observes in a field, are local and of exogenous origin. Even in these lesions, however, and especially on the leaves, the pathogene usually localizes or develops more rapidly in the xylem vessels.

The rapid development of the blight in a field depends considerably upon warm weather. It may be observed that the disease is more prevalent in hot seasons than in cool ones. In the greenhouse, infection is more easily obtained at 80° F. than at 65° F., and at the latter temperature the leaf spots develop very slowly. One observation in the field on the relation of temperature to the blight is possibly worthy of recording here. In 1922 the writer had a small field of beans at Ithaca on a northern slope.

Twice these beans were inoculated by spraying with a water suspension of *Phyt. phaseoli*, but the disease progressed very slowly. On a southern slope in the same field, and only 300 feet distant, a variety test of beans was being conducted. In this plot the blight broke out and spread rapidly from plant to plant, causing considerable damage. The difference in temperature in the two plots of beans was held accountable for the difference in the severity of infection in the two places. The pathogene progresses more rapidly in the plant tissue at high temperature than at low.

With the infection of the seeds of diseased plants, the life history of *Phyt. phaseoli* is completed. The organism gains entrance to the seed by passage through the vascular system or through local lesions on the pod. Seeds that are but slightly infected are more likely to introduce the organism into the field in the following season than are severely infected seeds. The latter are usually removed during the process of cleaning and picking in the seed houses, or, if planted, they may decay.

SYMPTOMS

General

Generalized symptoms may occur on the bean plant when the vascular system of the stem has become invaded by the pathogene. The severity of the symptoms varies with the proportion of the vascular system that is invaded. In light infections a stunting of the plant often will occur and will scarcely be noticeable except in comparison with normal individuals. On such lightly infected plants, an incipient wilting may take place during hot, dry days, but a recovery is always made in the evenings.

When the bacteria begin to invade the entire system a true wilt usually results. Seedling wilts are not infrequent, and at times this symptom arises very quickly. In older plants a leaf, a branch, or the entire plant may wilt (Plate V, D and E), and the progress of the wilt is usually in this order.

When seeds which are fairly severely infected, but not sufficiently so to injure the germination, are planted, a seedling may be killed as it emerges from the ground, or the bacteria may attack the growing tip of the plant and destroy it, leaving only the cotyledons. The appearance of such seedlings has given rise to the name *snakehead*. It should be stated here, however, that certain insects, fungi, bacteria, and seeds broken by threshers may cause a similar condition.

On the leaf

In the most typical leaf spots of beans due to *Phytomonas phaseoli*, shown in figure 1, the lesions may first appear as minute water-soaked spots or light green wilted areas, which, as they enlarge, dry out and become brown and brittle. About the edges of such lesions is a yellow

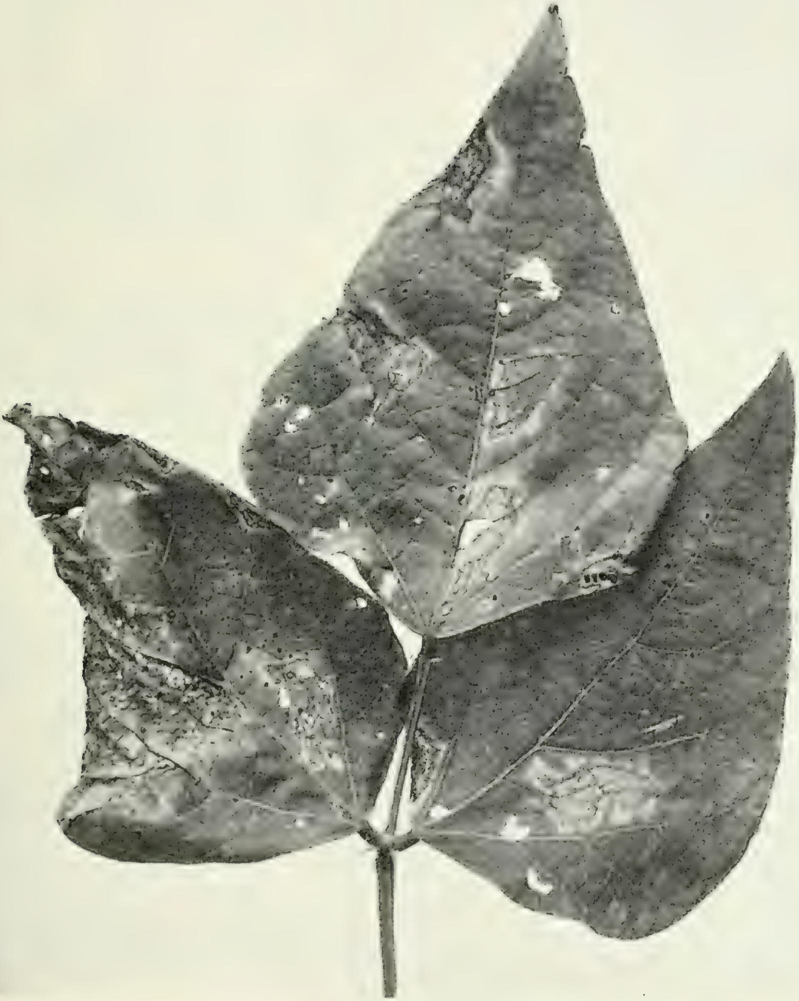

FIGURE 1. LEAF OF RED KIDNEY BEAN SHOWING LESIONS CAUSED BY PHYTOMONAS PHASEOLI

border, and surrounding this there is often a narrow pale green zone. One or more of these spots may increase in size and involve a large part of the entire leaflet. After a heavy rain or windstorm, such leaves have a very ragged and torn appearance. These typical lesions are, as a rule, due to external infection, but at times they may develop from the bacteria rising from the vascular system.

Several other types of leaf spots of beans have been observed and have been described before. One of these occurs only on young leaves. In this type the leaf is covered with small, angular spots which are at first water-soaked and dark green in appearance, but which later dry out and become brown. The necrotic tissue in the lesions is in the islets between the veins, and infection of the vascular bundles does not appear to have taken place.

On several occasions an anomalous type of lesion on the bean leaf has been observed. The varieties affected were the Red Kidney and an unknown green-pod variety. The spots in each case varied somewhat in size, but were not large, and were slightly raised above the healthy surface of the leaf (Plate II). In color they were a red-brown, and not unlike the leaf spot of lima beans due to *Phyt. vignae.* Isolations were made from a number of such lesions, and in all cases the pathogene appeared to be *Phyt. phaseoli.* No careful study of the cultures was made, however, outside of determining their pathogenicity on the common bean. Whether or not they were a distinct variety or race of *Phyt. phaseoli* is not known.

On the stem

Phyt. phaseoli produces water-soaked lesions on the stems of bean seedlings, as may be observed where diseased seeds have been planted. As these lesions grow older, they lose their water-soaked appearance, dry down, and become reddish brown in color. On older plants the water-soaked condition seldom is evident. Stem lesions usually are in the shape of streaks and are always longitudinal with the stem.

A second type of lesion found on the stem of the bean and due to this organism is the stem girdle. This injury sometimes causes considerable damage to the crop. The lesion is produced at the cotyledon, or more frequently at the first leaf node, or less frequently higher up on the plant. In these cases the vascular system is infected and the bacteria come to the surface in the leaf traces, resulting in the girdle. The color of these lesions is reddish brown, as is that of the streaks on the stem. Plants affected with the stem girdle become weakened at the infected node and are easily toppled over during a windstorm.

On the pods

Several types of lesions occur on bean pods, as may be seen in Plates II and IV and in figure 11 (page 46). The number of infections, the tenderness of the pod, and certain environmental factors, no doubt contribute in producing the differences in size and shape of the spots. All lesions are at first water-soaked and dark green in color, but later the tissues may become dry, sunken, and brick red. The process of drying begins at the outer edge of the spot and extends inward, and it is not uncommon to find a green, water-soaked lesion with a reddish, dry border. In many cases a yellowish crustation, due to the bacteria oozing out and drying over the lesion, covers the spot.

When the bacteria enter the pod by the vascular system, they may produce a long, irregular lesion along the suture (Plate IV, B). In other respects, however, the lesions so produced resemble those described above which arise from an external infection.

On the seeds

On white seeds, *Phyt. phaseoli* produces yellow spots of various sizes. When an entire seed is involved, it may be wrinkled and yellow, and appear as if it had been varnished. On colored seeds, small lesions are impossible of detection, and larger ones cannot be distinguished from anthracnose spots or abnormalities due to frost injury. Likewise, when immature seeds are harvested they may show a slight wrinkling and discoloration which at times could be mistaken for a bacterial infection.

If the bacteria enter the seed through the vascular system, and infection is light, they may produce only a yellow spot at the hilum, but in more severe cases they spread out underneath the seed coat. Seed slightly infected, either at the hilum or on the side, may escape detection during picking in the seed house and be used the following year for planting.

INOCULATION EXPERIMENTS

On the bean

Many of the symptoms just described have been reproduced on plants in the greenhouse by artificial inoculation, especially the symptoms connected with systemically infected plants. Red Kidney was the variety used, and the temperature maintained was approximately 80° F. More success in producing a vascular infection resulted from employing recently isolated strains of the pathogene than with strains that had been in culture for a year or more. Plants that were inoculated by pricking at the cotyledon node and were then placed in a temperature of 65° F. did not wilt as did those shown in Plate V, D and E, which were at 80° F. The plants in the lower temperatures, however, developed typical leaf spots on the lower leaves, and the bacteria in such lesions could be traced down the xylem vessels to the point of inoculation on the stem. When plants were inoculated through injuries, positive results were obtained in 100 per cent of the cases. When plants were sprayed with a water suspension

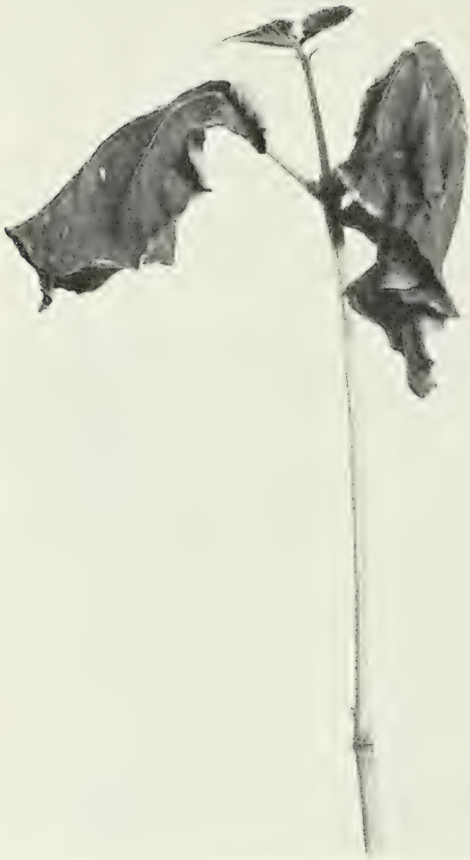

FIGURE 2. WILT OF BEAN SEEDLING CAUSED BY PHYTOMONAS PHASEOLI

The seedling was artificially inoculated at the cotyledon node

of the bacteria, and no mechanical injury was made in the plants, the results were variable. Environmental conditions favorable for infection under such circumstances are not yet sufficiently understood.

Cross inoculations

Inoculation experiments were conducted on a number of legumes which are listed in table 3 (page 77). On the sieva bean (*Phaseolus lunatus* L.), the scarlet runner bean (*P. coccineus* L.), and the hyacinth bean (*Dolichos lablab* L.), the symptoms produced by the disease were very similar to those on the common bean (*P. vulgaris* L.). Good infection was obtained also on the white-flowering lupine (*Lupinus polyphyllus* Lindl.). With this species, inoculations were made by pricking the crown and introducing the organism. A wilt of the leaves and the petioles resulted, and the pathogene was easily recovered from the leaves. Gardner (1924) reported *Strophostyles helvola* (L.) Britton as a host of *Phytomonas phaseoli*, but this species was not used in these experiments.

In 1924 the writer reported some inoculation experiments on various species of legumes grown in the garden with some varietal susceptibility tests of *P. vulgaris* (Burkholder, 1924). In the light of the present series of carefully conducted experiments in the greenhouse, the former inocula-

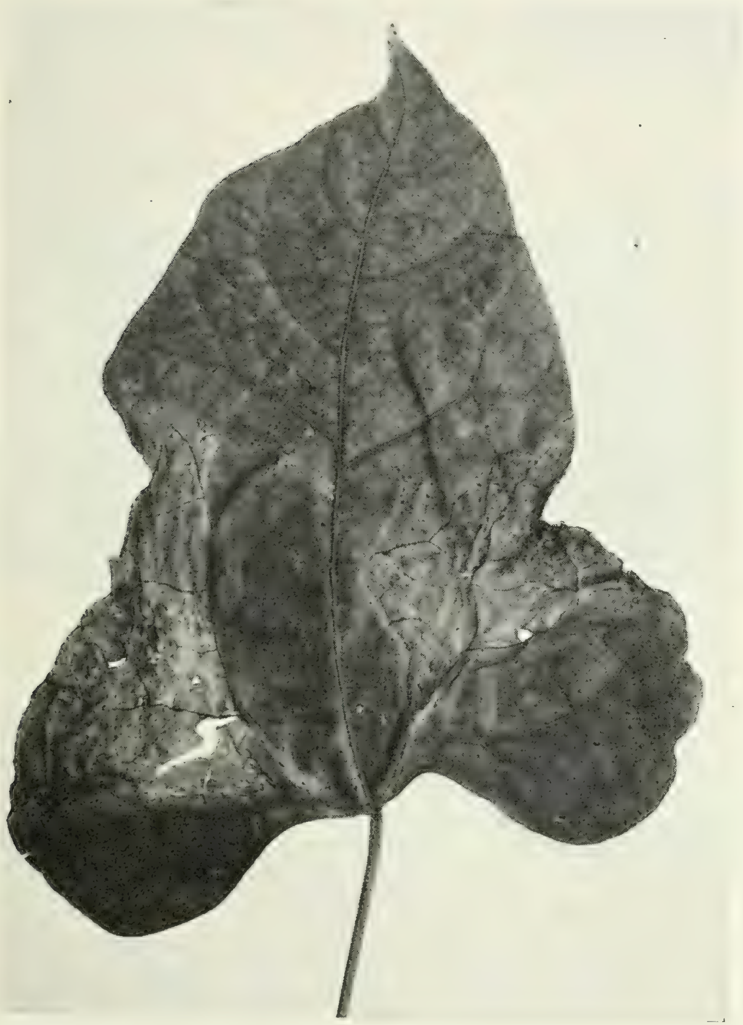

FIGURE 3. PRIMARY LEAF OF A BEAN PLANT SHOWING LESIONS RESULTING FROM INOCULATING THE STEM OF THE PLANT WITH PHYTOMONAS PHASEOLI

tion tests do not appear in all cases to be trustworthy. In these latter experiments, negative results always were obtained on the following species: adzuki bean (*P. angularis* Wight.), cowpea (*Vigna sinensis* Endl.), yard-long bean (*V. sesquipedalis* W. F. Wight.), soybean (*Glycine max* Merr.). kudzu vine (*Pueraria hirsuta* Schneid.), broad bean (*Vicia faba* L.), field pea (*Pisum sativum* L. var. *arvense* Poir.), sweet clover (*Melilotus alba* Desr.), and alfalfa (*Medicago sativa* L.).

ETIOLOGY

Many strains of *Phytomonas phaseoli* have been isolated, from time to time, from various types of symptoms and from various parts of the bean plant (*Phaseolus vulgaris*). Most of these strains have been from specimens gathered in New York, but in two cases they have been from foreign countries — one from Bermuda and one from Switzerland. Besides being isolated from *P. vulgaris*, cultures have been obtained from *P. lunatus* and *P. coccineus*. In all these instances the strains have been practically identical, which indicates that *Phyt. phaseoli* is a very stable species.

In culture the organism does change somewhat. While it still may produce as vigorous a growth on agar, certain biochemical reactions in culture appear to diminish though they have never been observed to be entirely lost. Strains that have been grown in culture for a number of years will not liquefy gelatin as rapidly as will recently isolated strains, and, similarly, the action on milk is delayed. Consequently, in comparing old and young cultures there frequently are shown minor differences which would not exist if the strain had been under identical conditions previously.

Morphology

Phyt. phaseoli is a medium-sized rod with rounded ends, occurring singly or in pairs. Filaments have never been observed in this species. Often certain cells appear to be oval, but in cultures twenty-four hours old the variation about the mode is not great. Such cultures, on beef-extract agar (pH 6.9) and incubated at 27° C., show the following dimensions: 1.9μ (1.35 to 3.6μ) by 0.87μ (0.45 to 1.35μ).

The organism is motile by means of a single polar flagellum. It is Gram-negative, not acid-fast, and capsule production has not been demonstrated. When grown on a medium containing dextrose a large amount of gum is produced. The pathogene appears to be a fairly strict aerobe.

Cultural characteristics

(All cultures incubated at 27° C. unless otherwise stated)

Beef-extract agar slants (pH 7.0). A moderate growth develops along the streak within twenty-four hours. Growth becomes abundant, filiform, wet, glistening, and amber yellow in color. The consistency is often very watery. From appearance the cultures are not amorphous, since various shades and intensities of the pigment occur within the bacterial growth.

Beef-infusion agar slants (pH 7.0). Growth does not vary to any appreciable extent from that on beef-extract agar. Considerable difficulty has been experienced, however, in keeping this species alive on this agar. It often dies within a week's time. This inability to remain viable on beef-infusion agar was noticed with several different strains of the pathogene.

Beef-extract agar plates (pH 7.0). Seven-days-old colonies are circular, approximately 3 to 5 millimeters in diameter, glistening, and primulin yellow in color. The consistency is watery to butyrous. The edges are entire, and their color is not so intense as that of the center of the colony.

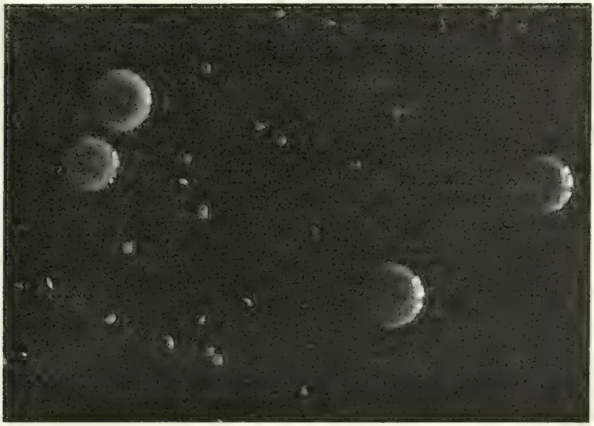

FIGURE 4. SEVEN-DAYS-OLD SURFACE AND DEEP COLONIES OF PHYTOMONAS PHASEOLI ON BEEF-EXTRACT AGAR (pH 7.0) AT 27° C. × 2

Beef-extract bouillon (pH 7.0). The broth becomes cloudy in twenty-four hours, and very cloudy in forty-eight hours. A yellowish ring is formed, and later a heavy sediment.

Gelatin stab (pH 6.6). (Temperature 21° C.) Growth occurs along the stab within twenty-four hours. On the second day slight liquefaction may be observed, which proceeds slowly until it is complete in approximately five weeks. Various types of liquefaction occur at different times, with different strains, and in different lots of gelatins.

Milk. Growth is first observed in about four days, when the medium begins to clear very rapidly. A yellow surface layer is formed, and a more or less heavy sediment appears at the bottom. Within several weeks the upper half of the tube is a clear yellowish liquid, the lower part is cloudy, and a great many tyrosine crystals are formed in the digesting casein. The medium is rendered alkaline.

Litmus milk. Growth is very similar to that in plain milk, with the exception of the color reaction. Litmus is reduced, although there is always a trace of purple in the tube. At the end of six weeks the upper part is a slightly reddish liquid, while the lower part is a muddy yellowish brown medium with crystals.

Brom cresol purple milk. The medium soon becomes a rather clear reddish purple liquid with a muddy sediment. As with all milk cultures, tyrosine crystals are formed in abundance.

Uschinsky's solution. A very faint clouding occurs in about ten days. Within two weeks there is a fair cloudy growth, but no further change takes place.

Fermi's solution. As a rule there is no growth. In a few cases a light cloudy growth has been observed.

Cohn's solution. There is no growth.

Potato cylinders. In twenty-four hours there appears a very good watery growth along the streak, which is primrose yellow in color. The growth soon covers the entire surface, and the potato cylinders begin to dissolve into a rather slimy mass. This breaking-down of the potato is one of the striking peculiarities of this pathogene. The color of the mass soon becomes a reed yellow.

Cellulose digestion. A light cloudy growth appears in the broth in twenty-four hours. Within a week or two, a yellow color may be noticed on the filter paper just above the surface of the liquid and at the end of the paper an inch or so above the liquid. There is no digestion of the cellulose.

Starch agar plates (pH 6.6). Plates streaked five or six times, and tested with a saturated solution of iodine in 50-per-cent alcohol, show the starch to be practically all digested at the end of two days.

Dextrose broth. A cloudy growth occurs in twenty-four hours in the open arm of a fermentation tube. No acid nor gas is produced.

Saccharose broth. A cloudy growth occurs in twenty-four hours in the open arm of a fermentation tube. No acid nor gas is produced.

Lactose broth. A cloudy growth occurs in twenty-four hours in the open arm of a fermentation tube. No acid nor gas is produced.

Maltose broth. A cloudy growth occurs in twenty-four hours in the open arm of a fermentation tube. No acid nor gas is produced.

Indol production. The organism grows well in trytophane broth, and determinations made at the end of one and of four days with the Ehrlich-Böhme test show no indication of indol production.

Nitrate reduction. The pathogene does not reduce nitrates to nitrites. Tests made at the end of two, four, and eight days have all been negative. The method used was that of sulfanilic acid and a-naphthalamine in acetic acid.

Hydrogen-sulfide production. The pathogene does not grow on Wilson's medium. On lead-acetate agar there is a good growth along the stab and on the surface of the agar. A very slight browning takes place along the stab. If hydrogen sulfide is produced, which is contrary to other reports, there is only a slight production.

Chromogenesis. The distinct yellow pigment appears to be produced on all types of media. In liquid media, yellow rings and yellow sediments are distinctly evident. The pigment is not water-soluble.

Oxygen relations. From its behavior in fermentation tubes and in shake agar cultures, the organism appears to be a rather strict aerobe.

Longevity. In a dry condition, on or in the seed of its host plant, the pathogene will remain viable for several years. In culture, however, the life of the organism is comparatively short. Transfers should be made at least once a month to prevent the strain from dying out.

Virulence. It has been stated in literature that the organism does not lose its pathogenicity or its virulence in pure culture. The reverse statement also has been made. In working with a great many strains, the writer has always been able to produce lesions on green pods no matter how long the strain had been in culture. In the endeavor to produce a seedling wilt or to cause a systemic infection of a bean plant, success appears always to be certain with a recently isolated strain, but negative results are often obtained with a strain that has been in culture for a year or two. Possibly this accounts for the difficulty obtained from time to time in producing an infection of the vascular system.

Thermal death-point. A few thermal-death-point tests have been made with this species, but with indefinite results. The thermal death-point appears to be low, probably around 48° C.

Brief description

Phytomonas phaseoli is a medium-sized rod with rounded ends, occurring singly or in pairs. The average length of the cells is 1.9μ. The organism is motile by means of a single polar flagellum. It is Gram-negative, not acid-fast, and apparently a fairly strict aerobe. Capsules have not been observed.

On agar slants the growth is good, filiform, and yellow in color. The consistency of the culture is watery to butyrous; broth cultures are cloudy in twenty-four hours; gelatin is liquefied; milk is peptonized without a curd; there is no acid nor gas in dextrose, saccharose, lactose, or maltose broth; starch is digested; nitrates are not reduced to nitrites; indol is not formed; hydrogen sulfide is not produced or but slightly; there is no growth in Fermi's or in Cohn's solution; growth in Uschinsky's solution is slight; the thermal death-point is approximately 48° C.

The organism is pathogenic on the common bean (*Phaseolus vulgaris*) and on several closely related species.

A DISEASE OF THE BEAN CAUSED BY A VARIETY OF PHYTOMONAS PHASEOLI

(Var. *fuscans* n. var.)

In August of 1924, specimens of diseased bean leaves were collected in a garden at Montreux, Switzerland. The variety of bean was unknown, but it was of the green-pod type; and the lesions on the leaves of the plants were typical of those caused by *Phytomonas phaseoli.* The spots varied in size, were brown and necrotic, and were surrounded by a yellowish border. In December of the same year, attempts were made to isolate an organism from these specimens although the leaves were in a very dry condition. A part of a lesion was macerated in sterile water and dilution plates were made. In all the plates, yellow, rapidly growing bacterial colonies appeared, very much like *Phyt. phaseoli* in appearance except for one characteristic. The agar medium about all the colonies became, after a week's time, a distinct brown in color. It was thought at first that the organism produced a water-soluble brown pigment which dissolved throughout the neighboring medium. This interpretation, however, was found later to be incorrect. Further dilution plates were made with the pathogene to separate it from any contaminating organism; a single colony was transferred to a tube and its pathogenicity on the bean was proved. A reisolation then was made from one of the infected plants, and the culture was saved for further work.

In August, 1927, green-bean pods were obtained in a market at Lucerne, Switzerland, which showed dark green, water-soaked spots apparently due to a bacterial invasion. Later, from these spots an organism was isolated similar in appearance to the one just described. The colonies were yellow and the surrounding medium became a distinct brown in color. This second isolation of this bacterium appeared to be an indication that the pathogene was not uncommon in Switzerland, and from the nature of the lesions observed it caused a disease similar to the blight due to *Phyt. phaseoli.* The pathogenicity of this organism was tested by inoculating it into a bean plant, and after infection took place it was reisolated and saved for further work.

Miss Florence Hedges informed the writer that she also had isolated what appeared to be the same organism from diseased bean seed which had been imported from South America. The pathogene probably is widespread, but, owing to its similarity to *Phyt. phaseoli,* has been overlooked.

INOCULATION EXPERIMENTS

On the bean

Inoculation experiments were conducted on Wells' Red Kidney plants in a greenhouse with a temperature of approximately 80° F. While the organism never completely lost its pathogenicity in pure culture, its virulence at times appeared to wane and reisolations were deemed necessary. During the progress of the work a few infected plants were continuously on hand in the greenhouse so that reisolations could readily be made. Inoculations on seedlings made by pricking the stem at the cotyledon node usually caused a wilting of the plant. The incubation period varied; as a rule it was about a week, but in some cases the time was longer. There were no peculiarities of this wilt of seedlings to distinguish it from such a symptom produced by any of the other xylem-infecting organisms. The seedling simply wilted, although, as a rule, rather slowly.

When young plants past the seedling stage were inoculated, certain characteristic symptoms did occur. Few of the plants died, though many do which are infected with *Phytomonas phaseoli*, *Phyt. flaccumfaciens*, or *Phyt. medicaginis* var. *phaseolicola*. A leaf or perhaps a tip of a branch or a young pod of the inoculated plant would wilt and die, but the plant as a whole would struggle on. At the point of inoculation, where the tissue of the stem was injured, a necrotic lesion would develop. This lesion extended slowly up the stem, and to a less degree downward. The lesions were reddish brown in color, and were dry; and cracks longitudinal with the stem would sometimes appear. In some cases there was a slight hypertrophy of the tissue about the injury. This symptom rather distinguishes this disease from others of its type.

Infection may be obtained on pods of the Red Kidney bean if a water suspension of the pathogene is sprayed or painted on them. If the pods are injured, infection takes place more readily and with greater certainty, although injury is not necessary. The first symptom of the lesion on the pod is a small water-soaked pustule, which, however, soon breaks down and a green water-soaked spot is formed. This condition persists for some time, but later the lesions dry out and become brick red and slightly sunken. When artificially inoculated pods are gathered in the greenhouse and stored in envelopes in the laboratory, the lesions remain green in appearance and rather gummy. In this respect it does not vary greatly from the spots produced by *Phyt. phaseoli*. The early formation of a pustule is the only difference. Inoculation of the pod at the peduncle has resulted in water-soaked lesions along the suture (Plate IV, C). These are indistinguishable from such lesions produced by *Phyt. flaccumfaciens*. It was thought that some of the lesions on the stem and the pods had a brownish coloration, which was lacking in symptoms due to the other bacteria. In other instances, however, this color symptom was entirely absent. Lesions produced on the leaves of Red Kidney plants in the greenhouse could not be distinguished from those produced by *Phyt. phaseoli*. They were in appearance similar to those found in Switzerland.

In a few cases, young plants inoculated with this pathogene and left in a greenhouse at a temperature of 65° F. did not develop the disease. Other plants, however, of the same lots showed unmistakable symptoms when placed in a temperature of 80° F. A high temperature seems necessary for the development of the disease.

Cross inoculations

Besides inoculation of the common bean (*Phaseolus vulgaris*), infection was obtained also on the sieva bean (*P. lunatus*) and the scarlet runner bean (*P. coccineus*). On the sieva bean, definite necrotic lesions on the leaves followed inoculation of the stem. This was more likely to be the result than a primary wilting. In these instances the bacteria could be traced up the xylem vessels in the stem to the lesions on the leaves. Water-soaked lesions were obtained also on the pods. On the scarlet runner bean, the lesions were, as a rule, local about the point of inoculation, and caused only a slight injury to the plant as a whole. They were usually reddish in color, and extended a half inch or more up and down the stem.

Negative results were obtained with the following species: hyacinth bean (*Dolichos lablab*), white-flowering lupine (*Lupinus polyphyllus*), kudzu vine (*Pueraria hirsuta*), cowpea (*Vigna sinensis*), yard-long bean (*V. sesquipedalis*), soybean (*Glycine max*), broad bean (*Vicia faba*), field pea (*Pisum sativum* var. *arvense*), and sweet clover (*Melilotus alba*). On the green pods of the cowpea a small pustule sometimes formed, as though infection were going to take place, but this vanished shortly, with no further sign of the disease. The bacteria introduced as the inoculum evidently caused a slight disturbance, but, since they were unable to establish themselves and develop, the slight lesion did not persist any longer than the introduced bacteria did. Several attempts were made with this organism to obtain infection of seedlings of the hyacinth bean and the white-flowering lupine, since *Phyt. phaseoli* readily produced diseases in these two species. Under the conditions of the inoculation experiments, however, the organism under observation remained non-pathogenic. The host range of the two pathogenes appears to be different.

ETIOLOGY

Morphology

The pathogene, when grown on beef-extract agar (pH 6.9), is a fairly large rod with rounded ends, occuring singly or in pairs. Chains are seldom found, but during the early hours of growth long filaments are not uncommon. Individual cells stain well with carbol fuchsin and gentian violet; granules are observed at the center of the rod, or in some cases they may be polar. A culture twenty-four hours old on the above medium, grown at 27° C., showed the following measurements: 2.34μ (1.35 to 4μ) by 0.98μ (0.6 to 1.35μ).

The organism does not form spores, and capsules have not been demonstrated. When grown on a medium containing dextrose, a large amount of gum is produced. The organism is motile, but those cultures with which the writer has worked have not been actively so. One polar flagellum has been demonstrated by the use of Loeffler's stain.

Cultural characteristics

(All cultures incubated at 27° C. unless otherwise stated)

Beef-extract agar slants (pH 7.0). In twenty-four hours there is fair yellow growth along the streak, which by the third day becomes abundant, filiform, wet-slimy, and mustard yellow in color; the color is denser in the center and is pale and watery at the edges. The consistency of the culture is frequently watery to thin butyrous. On the second day a slight purplish color is noticeable in the medium. This gradually deepens through a brownish purple to a deep brown. The rate of formation of this pigment appears to vary somewhat with the medium.

Beef-infusion agar slants (pH 7.0). Growth is similar to that on beef-extract agar. The coloration of the agar varies with the lot of media used, from a light to a very deep brown. On one lot which colored very rapidly, the viability of the organism was lost within a week.

Beef-extract agar plates (pH 6.9). Colonies two weeks old are approximately 1 centimeter in diameter, are honey yellow in color, and often have a zone of a watery appearance about the edge. The edges are entire to slightly undulated. In consistency the colonies are watery to weakly butyrous. The yellow pigment of the cells appears somewhat dull, owing to the brown coloration of the medium.

Beef-extract bouillon (pH 7.0). The broth becomes cloudy in twenty-four hours. At the end of four days a light yellow ring develops and a slight flocculation appears in the medium. Small bacterial islands float on the surface and a yellow sediment is formed. Within a week the broth becomes brown near the surface and the color gradually extends downward. At the end of three weeks the medium is a dark brown throughout and the cloudiness of the culture clears to a considerable extent.

Gelatin stab (pH 6.6). (Temperature 21° C.) In twenty-four hours there is a light growth along the stab. Liquefaction begins in three days but is not complete until the end of five weeks. No consistent form of liquefaction occurs. At the end of seven days a browning of the liquefied medium takes place and the pigment progresses with liquefaction. A heavy yellow sediment is formed.

Milk. A yellow ring is noticeable in four days. In six days the milk is yellowish, and a clearing begins at the surface and proceeds rapidly throughout the tube. A heavy casein sediment is formed in the bottom, which is gradually digested. The liquid above is yellow and fairly clear, but later a brown color begins to appear at the surface of the medium and extends downward. After six weeks or more a careful examination will reveal small masses of tyrosine crystals which appear to be partly digested.

Litmus milk. A yellow ring is formed in four days, and a clearing of the milk begins at the surface and rapidly progresses downward. Litmus is reduced, and the liquid becomes rather muddy but later clears and is purplish in appearance. The brown pigment is then formed. At the end of six weeks the upper half of the medium is a clear purple-brown liquid, but the lower part is muddy in appearance.

Brom cresol purple milk. A yellow ring is formed in four days. On the fifth day a clear purple layer of liquid is produced at the surface, and on the eighth day the entire medium is clear and is a reddish purple by transmitted light. It is difficult to determine whether or not a brown pigment is formed. In six weeks the upper part of the tube is a clear wine-colored liquid but the lower part is muddy.

Uschinsky's solution. On the tenth to the twelfth day a light clouding appears in the medium. There is no further development at the end of six weeks.

Fermi's solution. There is no growth.

Cohn's solution. There is no growth.

Potato cylinders. In twenty-four hours a good watery growth, primrose yellow in color, appears along the streak and soon covers the entire surface. The growth appears slimy, and after a week's time the potato cylinders begin to dissolve. A slight brown color appears and the tuber assumes an olive-ocher color. At the end of five weeks certain tubes are almost black, while others are only a light brown. This is possibly owing to differences in cylinders from different potato tubers.

Cellulose digestion. A good growth appears in the broth, but there was no indication that the filter paper is attacked. A brown coloration appears both in the liquid and on the paper above the broth.

Starch agar plates (pH 6.6). Plates of starch agar streaked five or six times, and tested for starch digestion with a saturated solution of iodine in 50-per-cent alcohol, show a slight digestion of starch at the end of two days and an excellent digestion at the end of six days.

Dextrose broth. The broth in the open arm of a fermentation tube becomes cloudy in twenty-four hours, and later excellent growth is produced. At the end of seven days the brown coloration is distinctly noticeable. No acid nor gas is produced.

Saccharose broth. There is a light cloudy growth in twenty-four hours. A brown color appears within ten weeks. No acid nor gas is produced.

Lactose broth. In twenty-four hours there is a light cloudy growth in the open arm of a fermentation tube. A brown color develops later. No acid nor gas is produced.

Maltose broth. There is a cloudy growth followed by the production of a brown color in the open arm of a fermentation tube. No acid nor gas is produced.

Czapek's agar. An abundant, watery, pale yellow growth is produced, but no brown color is formed in the medium. The production of gum is very noticeable.

Shake agar culture. When a beef-extract agar with 1 per cent of glucose (pH 7.0) is used, there is only a surface growth and no colonies are formed in the depth of the medium.

Indol production. A good cloudy growth is produced in trytophane broth. At the end of one and of four days, tests made for indol by the Ehrlich-Böhme method show no trace of this substance.

Nitrate reduction. A good cloudy growth is produced in nitrate broth but nitrates are not reduced to nitrites. Tests were made at the end of two, four, and eight days with sulfanilic acid and a-naphthalamine in acetic acid. The brown pigment is formed in this medium.

Hydrogen-sulfide production. No growth is obtained on Wilson's medium. On lead-acetate agar a browning occurred along the stab, but this brown coloration appeared to be the usual coloration produced by this organism on most media. It is doubtful whether hydrogen sulfide is produced.

Chromogenesis. The brown water-soluble pigment which is formed in most media, owing to the action of the pathogene, is so striking in appearance that it demands a certain amount of attention. It is a phenomenon not commonly met with among the members of the bacterial plant pathogenes, although it occurs not infrequently with species of the genus Actinomyces. A brown pigment has been reported in literature as being produced by *Phytomonas campestre* and *Phyt. hyacinthi*, but most descriptions of these organisms do not refer to it; and a sufficient description has never been given to permit of determining whether or not it is similar to the pigment formed in media by the action of the bean pathogene. It is possible, too, that only certain strains of the two bacteria referred to are capable of causing the formation of the brown coloration of the media. The information is rather scanty.

The brown coloration formed in cultures of certain species of Actinomyces appears to be the same or a closely related product. With this group of organisms the phenomenon is considered to be the result of an enzymatic action on the amino acid, tyrosine, contained in the media, and the production of melanin. It is probable that two enzymes produced by the organism are implicated in the process. Waksman (1926) has reviewed the literature on tyrosinase and has suggested the possible chemical reactions which result in the brown substance formed. It will be noted in the above cultural descriptions of the organism that the brown color does not appear in synthetic media where tyrosine would be lacking.

Acting on this suggestion, Conn's (1921) synthetic medium for growing species of Actinomyces was prepared. In one lot of this medium, tyrosine replaced the sodium asparaginate. The organism grew fairly well on the two simple media, and on the second day a purple-brown color appeared in the tyrosine agar and deepened to a dark brown. No color appeared in the sodium-asparaginate agar. For further confirmation, *Phyt. phaseoli* was grown at the same time on the two media. After three weeks of incubation a light brown color did appear on the tyrosine agar, but this was considered to be due to a natural breaking-down of the amino acid under these conditions. One-tenth of 1 per cent of tyrosine had been used in this medium, which, no doubt, is an excessive amount. The difference in the two cases, however, was distinct. The organism under consideration produced a deep color, which began to be formed on the second day of incubation. Color did not appear in cultures of *Phyt. phaseoli* until after three weeks.

A further experiment, similar to the one just described, was conducted, using, in place of Conn's medium, the synthetic medium suggested by Ayers, Rupp, and Johnson and modified in the *Manual of Methods* of the Society of American Bacteriologists (1923–29). Dextrose was used as the sugar. There was no substitution in the nitrogen source, but to one lot of media 0.1 per cent of tyrosine was added. In no case, however, did a brown color develop on the agar. It is possible that there was an inhibiting factor present. Just what this inhibition is, was not determined, although it has been noticed that in beef-extract agar to which dextrose has been added, the color is somewhat slow in developing, and the amount is never great. This sugar may be accountable for the failure of the brown color to appear in the above-named synthetic medium.

That this brown water-soluble pigment in the various media is due to the action of an enzyme or enzymes upon tyrosine seems evident, however, from the following facts: First, the color appears in media containing peptone, potato, or some other substance, where the tyrosine would probably be present, and in milk where it could be formed. Secondly, no brown color develops in a synthetic medium in the absence of tyrosine. Thirdly, such a pigment develops in Conn's medium when tyrosine is substituted for sodium asparaginate. Fourthly, in milk cultures that have begun to show a brown pigment, masses of tyrosine crystals may be found which are partly digested. It is probable that the pathogene, acting upon casein, produced tyrosine, which in turn is acted upon by a further enzyme or enzymes.

Longevity. No carefully planned experiments have been conducted on this phase of the problem, but certain observations are of interest. On beef-extract agar and broth, many of the strains die within a month. Cultures usually are transferred every two to three weeks to insure their remaining viable. The browning and clearing of broth cultures often is accomplished by death. On beef-infusion agar, where the brown pigment appears early and becomes intense, many cultures die within a week. There appears to be a correlation between the production of the brown color and death of the culture. Old potato cultures, however, have revealed viable organisms.

Nomenclature

Although the production of this brown pigment in various media is a very striking character of the pathogene, it is the only cultural characteristic so far noted which separates the organism from *Phyt. phaseoli.* The formation of filaments is a slight, but distinct, morphological character, and in the symptomatology and in the host range there are several differences, the last two distinctions of which have already been discussed. It is felt that these differences are probably varietal and not specific. Therefore the name *Phytomonas phaseoli* var. *fuscans* n. var. is given to the pathogene.

Brief description

Phytomonas phaseoli var. *fuscans* is a non-spore-forming rod with rounded ends, occurring singly or in pairs. The cells measure 1.35 to 4μ by 0.6 to 1.35μ. Filaments are not uncommon. The organism is motile by means of a single polar flagellum. It stains well with carbol fuchsin and gentian violet, showing granules. It is Gram-negative, not acid-fast, and an aerobe.

The colonies on nutrient agar are yellow, fairly rapid-growing, in many cases with a watery-appearing zone about the edge. The edges are entire to slightly undulating. The consistency of the culture is watery to butyrous; most media are colored brown; the broth is cloudy in twenty-four hours, a yellow ring is formed, and the medium becomes brown at the surface, the brown color extending gradually throughout; gelatin is liquefied and colored brown; milk is alkaline and cleared, and later a brown pigment is formed; there is no acid nor gas in dextrose, saccharose, lactose, or maltose broth; starch is hydrolyzed; nitrates are not reduced to nitrites; indol is not produced; hydrogen sulfide is not formed on lead-acetate agar; there is no growth on Wilson's agar, in Cohn's solution, or in Fermi's solution; growth in Uschinsky's solution is slight; the thermal death-point is approximately 49° C. The viability of the organism in culture is not great.

The organism is pathogenic on the common bean (*Phaseolus vulgaris*), the sieva bean (*P. lunatus*), and the scarlet runner bean (*P. coccineus*).

THE BACTERIAL WILT OF THE BEAN CAUSED BY PHYTOMONAS FLACCUMFACIENS

The bacterial wilt of the bean was reported first by Miss Hedges (1922), who later (1926) published a fuller account of the disease. Leonard (1923, 1924) also has reported on various phases of the wilt. The disease is of widespread distribution in the United States, and in certain sections causes considerable loss. In New York State, although it has been known for some time, it has never become of as great economic importance as have some others of the bacterial diseases. It does occur, however, generally throughout the sections growing dry shell beans, and may be found on such varieties as Red Kidney, Pea, and White Marrow.

LIFE HISTORY

In many respects the life history of *Phytomonas flaccumfaciens* is very similar to that of *Phyt. phaseoli.* There are, however, certain differences, since *Phyt. flaccumfaciens* is more limited in its activities on the host plant.

The overwintering of the bacteria takes place in the seed, and in this manner it is introduced into the field in the following year. There may be other methods by which the pathogene may survive the winter, but they are probably not important in its life history. The production of diseased plants from slightly infected seeds can be demonstrated easily, and infected seeds are sometimes frequent in seed beans. On the other hand, no work has been done to determine whether or not the bacteria can overwinter in the soil or in refuse. In a dry condition in the seed, and even in the leaves, of its host plants, the organism is extremely resistant. It has been isolated from dried leaves kept in the herbarium for five years, and from seed stored in the laboratory for a similar period of time. This duration of time is possibly not the limit of its viability period, but further tests have not been made. Infected seed treated at a temperature of 100° C. in a hot-air oven for an hour still yield pathogenic cultures.

The activities of *Phyt. flaccumfaciens* in relation to its host are as follows: When an infected seed germinates in the soil, the bacteria become active and pass from the cotyledons into the stem. Here they may be found in great numbers in the tissue of the xylem vessels, for which they show a decided preference. This organism, as well as *Phyt. phaseoli*, passes through an entire plant in the vascular system. Other tissue besides the xylem seldom is infected, and the organism appears to lack the ability to invade such tissue. In spreading from one bean plant to another, however, there is some difficulty. The bacteria can produce an infection only through mechanical injury, as Miss Hedges (1926) has shown. Consequently, rain splashing the bacteria from plant to plant would be of no importance unless some injury had already occurred in the plant that was spattered. In many bean fields, insects of both the leaf-chewing and the sucking types are found commonly, and probably are an important factor in the spread of the pathogene. Experimental evidence on this point, however, is lacking.

In completing their life history, the bacteria must again infect the seed. They do this, in a large majority of cases, by passing into the seed through the vascular system. The entrance of *Phyt. flaccumfaciens* through the side of the pod and the ultimate invasion of the seed, as is a common occurrence with *Phyt. phaseoli*, rarely happens. *Phyt. flaccumfaciens*, when introduced into the fleshy tissue on the side of the pod, does not cause infection, or, at the most, produces only a very slight lesion (Plate III, A). When the injury extends into the pod cavity, however, the bacteria may multiply there, although they cause scarcely any discoloration of the pod tissue. Under such conditions, bacteria have been observed to grow on the surface of a seed while the seed matured normally. If the seeds are injured during inoculation, they become infected and rot. Seeds infested with the bacteria on their surface may give rise to diseased seedlings.

When *Phyt. flaccumfaciens* enters the pod through the vascular system of the pedicel, a suture lesion usually is produced (Plate IV). Since otherwise this pod tissue does not become infected, the suture lesion appears to be the result of an overflowing of the bacteria from the xylem vessels.

SYMPTOMS

General

Since *Phytomonas flaccumfaciens* is primarily a vascular parasite and invasion has a decided tendency to become systemic in nature, the general symptoms of a diseased plant are the most pronounced. A stunting of an infected plant takes place and, as a rule, quickly develops into a wilt. It is this wilting of an infected plant, which is the most outstanding symptom of the disease, that led Miss Hedges (1926) in first describing the disease to give to it the common name *wilt*. At any stage in the development of the plant the wilt may take place, from a seedling wilt to the wilt of the entire plant bearing mature pods. Wilting of a part of the plant, as a branch, also is not uncommon. Since the stalk and the branches of a maturing bean plant are more or less woody in nature, the true flagging is limited primarily to the leaves and the young growing shoots. Even the leaf petioles seldom droop, but remain erect. Wilted leaves become dry and brown, and, after several rains, very ragged. Sometimes the leaves on an infected plant will not persist but will drop off.

On the leaf

As already stated, the wilt is the primary symptom of this disease, and this is especially true with reference to the leaves. The leaflets may flag, beginning at the tip or along the side. Such leaves fail to recover, dry up, and turn brown, still persisting on the plant. A heavy wind or rain may destroy these leaves completely or whip them to a very ragged condition. In some cases, but less frequently, only a part of the leaf may wilt and die, thus giving rise to a rather characterless lesion as shown in figure 5.

In some plants, the vascular systems of which are infected, the leaflets may turn somewhat yellow, dry up, and fall off. In such cases the main petiole remains standing erect.

On the stem

There is probably only one type of lesion found on the stem of bean plants infected with *Phyt. flaccumfaciens*. This is a stem girdle, or a disease of the nodes. These lesions appear to be of endogenous origin, the bacteria working their way out from an infected xylem along a leaf trace. Such infected nodes turn red, and are greatly weakened so that the plant is broken off easily during a windstorm. The stem girdle is not peculiar to this disease, but may be caused by *Phyt. phaseoli* and possibly by *Phyt. medicaginis* var. *phaseolicola*.

On the pods

Lesions produced on the pods by *Phyt. flaccumfaciens* occur along the suture. The bacteria spread from the fibrovascular bundles, causing a

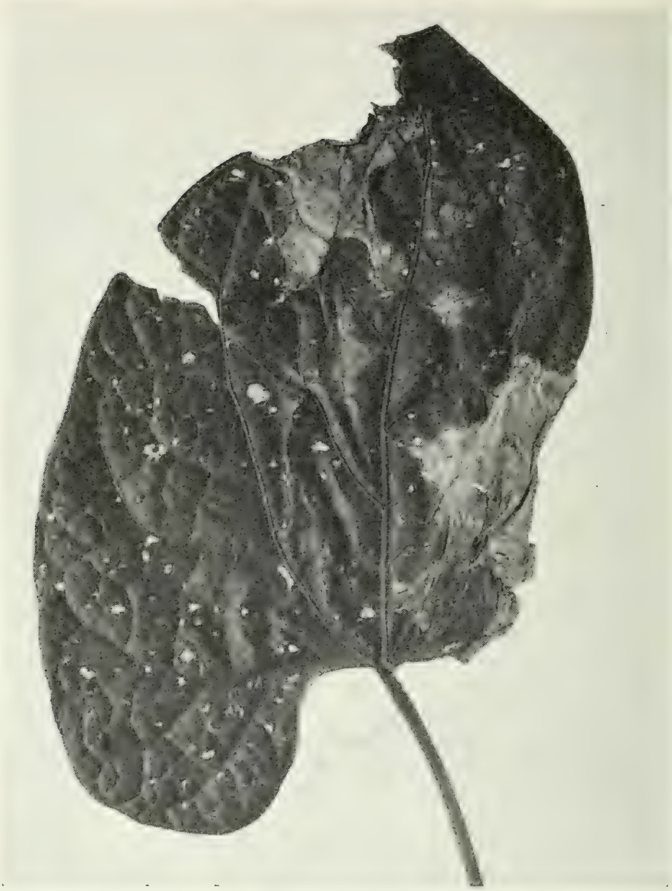

FIGURE 5. PRIMARY LEAF OF SIEVA BEAN (PHASEOLUS LUNATUS) SHOWING LESIONS RESULTING FROM INOCULATING THE STEM OF THE PLANT WITH PHYTOMONAS FLACCUMFACIENS

dark green, water-soaked spot sometimes extending the entire length of the pod (Plate IV). Lesions produced in the greenhouse retain this green, water-soaked appearance even after the remaining part of the pod has lost its chlorophyll, dried, and turned white. In such a state they appear dark green and rather gummy. It is not known whether or not the lesions under natural conditions in the field ever dry out and turn reddish brown, as do the water-soaked spots of *Phyt. phaseoli.* This point is yet to be determined.

On the seeds

The symptoms produced by *Phyt. flaccumfaciens* on the seeds of beans are practically the same as certain of those produced by *Phyt. phaseoli.* The bacteria entering the seed through the vascular system may produce a yellow spot on the hilum. If infection is more severe, the organisms spread out underneath the seed coat and discolor the seed. If the seed is white, infection with this pathogene may turn it a bright yellow. Severely infected seed may be very wrinkled and appear as if varnished.

INOCULATION EXPERIMENTS

All of the symptoms described above have been produced on the Red Kidney bean in the greenhouse by artificial inoculation. Infection always is obtained at a temperature of 80° F., provided the plant is injured and the injury extends into the fibrovascular bundles. Red Kidney is very susceptible and is one of the best varieties to use for inoculation purposes.

Cross-inoculation experiments were conducted on thirteen species of legumes, eight of which proved to be highly susceptible. *Phytomonas flaccumfaciens* thus appears to have a particularly wide host range, and there are, no doubt, other susceptible species which are still unknown. The inoculations were made by injuring a seedling or a young, vigorously growing plant on the stem, and introducing the pathogene at the same time. The symptoms resulting on the susceptible species were more or less similar to those appearing on the common bean (*Phaseolus vulgaris*); that is, a wilt occurs, either of the entire plant or of a part of it. *Phyt. flaccumfaciens* remained in all cases a vascular parasite. The susceptible species are as follows: sieva bean (*P. lunatus*), scarlet runner bean (*P. coccineus*), adzuki bean (*P. angularis*), cowpea (*Vigna sinensis*), yard-long bean (*V. sesquipedalis*), soybean (*Glycine max*), and hyacinth bean (*Dolichos lablab*).

Negative results were obtained with the following species: white-flowering lupine (*Lupinus polyphyllus*), kudzu vine (*Pueraria hirsuta*), broad bean (*Vicia faba*), field pea (*Pisum sativum* var. *arvense*), and sweet clover (*Melilotus alba*). Since infection always was obtained when susceptible species were inoculated, it is felt that this list is correct under the conditions used. When infection was not obtained at the first inoculation, as on these five species, the experiments were repeated two or three times. These inoculations involved from twenty-five to fifty individuals in all.

ETIOLOGY

Miss Hedges (1926) has already published a very excellent description of this organism, and the writer's description, presented here, is included principally to give opportunity for a comparative study with the other bacteria which cause blights on beans. Ten strains isolated from various places in New York State were used in preparing the following description.

It was found that certain strains seemed to vary from the original, and these variations are pointed out. *Phytomonas flaccumfaciens* appears not to be as stable a species as *Phyt. phaseoli*.

Morphology

Phyt. flaccumfaciens is a short rod with rounded ends, non-spore-forming, occurring singly or in pairs. The longest rods often show a slight curve, and a few of the cells appear to be larger at one end than at the other. Grown on beef-extract agar (pH 6.9) at a temperature of 27° C., the cells of a culture twenty-four hours old measured: 1.75μ (0.9 to 2.7μ) by 0.82μ (0.6 to 1.2μ). Granules have been observed in the cells.

The organism is motile by means of a single polar flagellum. It is not acid-fast, but is Gram-positive. One strain proved to be Gram-variable, and even grew well in gentian-violet agar. Capsules have not been observed.

Cultural characteristics

(All cultures incubated at 27° C. unless otherwise stated)

Beef-extract agar slants (pH 7.0). Growth appears in twenty-four hours. The growth is never more than moderate, is filiform, smooth, translucent, and straw yellow in color. The color appears to vary with the lot of media. In some cases there is scarcely any pigment production until after three or four days, and then it may be very weak. In other cases the pigment may be deep and similar to that of *Phyt. phaseoli*. The consistency is often viscid.

Beef-infusion agar slants (pH 7.0). Growth is similar to that on beef-extract agar, although sometimes it is more copious. The color is more intense on this agar, and becomes in several days a light cadmium.

Beef-extract agar plates (pH 7.0). Seven-days-old colonies are circular, approximately 3 to 5 millimeters in diameter, and mustard yellow in color. The consistency is slightly stringy.

Beef-extract bouillon (pH 7.0). The medium becomes slightly cloudy within twenty-four hours. Growth advances somewhat, but there is never more than a moderate cloudy appearance. A light sediment is formed.

Gelatin stab (pH 6.6). (Temperature 21° C.) Growth appears along the stab within twenty-four hours. Liquefaction varies with the strain, and may begin within three days to three weeks. In some cases liquefaction has been complete in five weeks. In certain lots of gelatin, several strains have shown no liquefaction at all.

Litmus milk. Litmus milk begins to turn pink on the fourth day. A distinct yellow ring is formed, and after a week's time reduction of the litmus commences. After four weeks a soft curd is formed, which becomes solid, and in some cases a slight peptonization takes place. Many variations of this procedure may occur: in litmus reduction, from complete to slight; in curd formation, from none to a solid; and in the amount of peptonization.

Brom cresol purple milk. A slight yellow-green color appears in about six days, and later a yellow ring is formed. At the end of four weeks, a very soft curd may appear and slight peptonization begins. At the end of eight weeks, the cultures are a greenish brown in color.

Uschinsky's solution. No growth was observed.

Fermi's solution. No growth was observed.

Colin's solution. No growth was observed.

Potato cylinders. As a rule, growth appears in twenty-four hours along the streak. It is a light to fair growth, and is pale yellow at first, deepening in seven days to a mustard yellow. The medium may or may not be darkened. Certain strains will scarcely grow on potato. One strain produced a vigorous growth covering the entire cylinder in five days, and was a deep aniline yellow in color. The medium remained unchanged.

Cellulose digestion. A very light, cloudy growth appears in the liquid, but there is no sign of cellulose digestion.

Starch agar plates (pH 6.6). Macroscopically there is no indication of starch digestion. Miss Hedges, however, reports that when the cultures are examined microscopically, a slight digestion may be observed. In making determinations, plates were streaked five or six times, and tests for digestion of starch were made at the end of two, six, and ten days. The tests were made by flooding the plates with a saturated solution of iodine in 50-per-cent alcohol.

Dextrose broth. A cloudy growth appears in the open arm of a fermentation tube within twenty-four hours. A color reaction due to acidity appears in two to three days. The intensity of the reaction varies with the strain, and in all cases the color appears to deepen with age. The following table shows the variation in hydrogen-ion concentration in dextrose broth due to ten different strains. The strains are listed in the order in which they were isolated.

Strain	Original pH	pH after seven days	pH after fourteen days
6	7.0	6.50	5.90
17	7.0	6.00	5.00
24	7.0	6.45	5.10
30	7.0	6.35	5.80
31	7.0	6.55	5.20
32	7.0	6.45	5.85
33	7.0	6.50	6.45
33–5	7.0	6.35	5.45
B	7.0	6.05	5.00
O	7.0	6.15	6.15

Saccharose broth. A cloudy growth appears in the open arm of a fermentation tube within twenty-four hours. The medium soon becomes acid, and the reaction becomes greater with age. No other change occurs.

Lactose broth. A light, cloudy growth appears in the open arm of a fermentation tube within twenty-four hours. On the second day the medium shows an acid reaction. From a light pink in color, it gradually deepens to a distinct red.

Maltose broth. A light, cloudy growth appears in the open arm of a fermentation tube within twenty-four hours. A slight acidity appears on the fourth day and gradually increases in intensity. No other changes occur.

Indol production. The organism does not produce indol when grown in trytophane broth, as shown by the Ehrlich-Böhme test used at the end of one and of four days.

Nitrate reduction. The organism grows well in nitrate broth, and tests made at the end of two, four, and eight days with sulfanilic acid and a-naphthalamine in acetic acid show no reduction of nitrates to nitrites.

Hydrogen-sulfide production. A very light growth is obtained on Wilson's medium, and in certain cases there has been no growth. Where growth does occur there is no positive reaction for hydrogen-sulfide production. Growth occurs in lead-acetate agar stabs with all strains tested. Certain strains show a slight browning along the stab, while others remain colorless. Hydrogen sulfide is not produced, or is produced only weakly by certain strains.

Chromogenesis. The organism is pale yellow in color, but the intensity of the pigment may vary with the strain and the medium used. The color is more intense on beef-infusion agar, and is almost absent on dextrose agar.

Oxygen relations. Although the organism ferments all the sugars tested, there is no sign of growth in closed arms of fermentation tubes. In shake cultures of beef-extract agar with 1 per cent of dextrose, colonies appear 5 millimeters below the surface. On the whole, the pathogene appears to be a fairly strict aerobe.

Longevity and virulence. This pathogene is very resistant to adverse conditions, and remains viable in culture for a long period of time. It has been isolated from dried leaves which have been in the herbarium for five years. Once in culture, it does not lose its pathogenicity or its virulence to any appreciable extent. Old cultures which are very dry will often yield viable cells. The organism may be recovered from bean seeds infected with it and treated with dry heat at 100° C. for an hour. It does not produce spores in culture, and probably does not in nature. Partly protected by the bean seed, and being in a dry condition, it is highly resistant to dry heat.

Thermal death-point. Tests have not been made to determine the thermal death-point, but Miss Hedges reports that it is approximately 60° C.

Brief description

Phytomonas flaccumfaciens is a short, non-spore-forming rod with rounded ends, occurring singly or in pairs. The average size of the cells is 1.75 by 0.82μ. The organism is motile by means of a single polar flagellum. It is Gram-positive, not acid-fast, and apparently a fairly strict aerobe. Capsules have not been observed.

On agar slants the growth is moderate, filiform, and pale yellow in color. The consistency of the culture is often viscid; broth cultures are cloudy in twenty-four hours; gelatin liquefaction, as a rule, is light; a soft curd is formed in milk; litmus is reduced; acid is produced, but no gas, in dextrose, saccharose, lactose, and maltose broth; starch is not hydrolyzed or but slightly; nitrates are not reduced to nitrites; indol is not formed; hydrogen sulfide is not produced; there is no growth in Uschinsky's, Fermi's, or Cohn's solution; pathogenicity or virulence is not lost in culture.

The organism is pathogenic on the common bean (*Phaseolus vulgaris*) and on many related species.

HALO BLIGHT CAUSED BY PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA[1]

The bacterial disease of greatest economic importance in New York is the one caused by the organism *Phytomonas medicaginis* var. *phaseolicola*. This disease was first noticed in 1924, and, because of the severity of its attack, suspicion immediately arose that it was distinct from the other known bacterial diseases of beans. The rapidity with which the disease spreads through a field, and the severity of the symptoms on the individual plants, are still undiminished.

In that section of New York where the Red Kidney bean is grown extensively, this disease has been in the past few years a limiting factor to production. It may be differentiated on this variety from the other bacterial diseases almost alone by the severity of its attack, regardless of the types of symptoms. On the other hand, the Pea bean, the Marrow, and the Yellow Eye, of the dry-shell varieties, and the Refugee of the green-pod types, are not so susceptible as the Red Kidney, although they should never be considered resistant.

The distribution of the disease, and the extent of its economic importance outside New York, have never been carefully investigated. Apparently it is of widespread occurrence. Miss Hedges (1928) has reported it from Georgia and Montana, and she states that in those sections it causes considerable losses to the crop. In 1927 the writer isolated the causal organism, *Phyt. medicaginis* var. *phaseolicola*, from specimens collected in Switzerland, showing that the disease it is not limited entirely to America. It probably is of widespread distribution, but its general similarity to the diseases produced by *Phyt. phaseoli* and *Phyt. flaccumfaciens* has caused it to be overlooked as a separate entity.

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[1] In a recent publication (Phytopath. 20:140. 1930), Miss Hedges states that *Phytomonas pueraria* is a synonym of *Phyt. medicaginis* var. *phaseolicola.*

The symptoms of the disease caused by *Phyt. medicaginis* var. *phaseolicola* have been described, but perhaps not fully. Careful observation of this disease in the field has been maintained during the past five years, and many variations of the symptoms have been noted. It has been observed that certain types of lesions, especially on the leaves of the host plant, occur in one season in profusion and in the following season are inconspicuous. These variations in symptoms appear to be due to ecological factors, and no indications have been observed that various strains of the pathogene are involved. Consequently, after these observations over a considerable period of time, a better picture may be given of the disease as it appears under natural conditions in the field than was given in the first report.

In the following descriptions of the symptoms of the disease, it must be remembered that observations have been made primarily in the field on the Red Kidney variety of beans. The symptoms do not differ, however, except in intensity, on such common varieties as Pea, Marrow, Yellow Eye, White Kidney, and Refugee. In the majority of cases the symptoms observed in the field under natural conditions can be reproduced in the greenhouse.

LIFE HISTORY

The life history of *Phytomonas medicaginis* var. *phaseolicola* is very similar in detail to that of *Phyt. phaseoli*, and for this reason only the salient points are given here.

The organism overwinters on and in the bean seed, and becomes active in the spring when such seeds are planted and have germinated. Sufficiently high temperature and enough moisture are necessary for the abundant development of the bacteria in the cotyledons. Here they multiply rapidly in the host tissue, and may serve as inoculum to be splashed by rain to the leaves and other parts of the infected individual or to near-by plants. The organism evidently gains entrance to the plant through the stomates, since mechanical injury is not necessary for infection to take place. A second mode of invasion is for the bacteria to enter the plant from the cotyledons by way of the vascular system. Under such conditions an infection of the vascular system takes place, the xylem being the tissue most generally invaded although the bacteria may break forth into the adjacent cells and even to the surface of the plant. Since *Phyt. medicaginis* var. *phaseolicola* exhibits a greater virulence than does *Phyt. phaseoli*, the disease from this last-named type of invasion is more likely to become acute, rather than remain dormant for some time as it does with *Phyt. phaseoli*. A further difference between the two organisms is that *Phyt. medicaginis* var. *phaseolicola* appears to produce a toxin which precedes the organism in its harmful effect on the plant. This is not so noticeable with *Phyt.*

phaseoli. The two organisms are alike, however, in the tissue which they attack. Each shows a preference for the vascular tissue, and uses the xylem vessels as a means of spreading throughout the plant. They can, on the other hand, attack the other tissues of the plant with great vigor, although they do not spread so rapidly in these.

One point in the life history of the organism of which little is known is its ability to overwinter in the soil. In the greenhouse it is known to live for several months in garden loam, but no carefully conducted experiments have been carried on in the open. From observations in the fields of New York, it is evident that clean seed, such as is brought in from California, produces, as a rule, healthy crops of beans. Since the crop rotation in the bean section, however, is seldom less than three years, it would be necessary for the pathogene to remain viable in the soil for that length of time in order for this phase of its life history to be of any economic importance. There is no indication that the organism possesses this ability.

SYMPTOMS

Stunting and wilt

It has been pointed out, in the discussion of the life history of *Phytomonas medicaginis* var. *phaseolicola*, that this pathogene, while not obligatively a vascular parasite like *Phyt. flaccumfaciens*, nevertheless does show a decided tendency to invade the xylem vessels. The result of the infection of the xylem tissue is, in most cases, a symptom complex, and should be regarded as such rather than as isolated individual symptoms. The latter is the case only when the infection is local. The symptoms arising from the infection of the xylem are definite, and in certain respects are peculiar to this disease. A seedling wilt, however, may result which is not peculiar to this organism and which cannot be distinguished from such a wilt induced by other bacterial pathogenes. In inoculation experiments this wilt takes place in about four days, but it is not of frequent occurrence in the field. Possibly the reason for this is that the seeds which are planted have been graded in a seed house, where the severely infected seed are removed and only the lightly infected ones remain; consequently, the bacteria are not present in sufficient quantity to enable them to reach the seedling from the cotyledon and cause a wilt at this early stage of development. Young plants with infected xylems, however, will soon show certain definite symptoms of the disease. They are stunted in growth, and as the pathogene progresses upward in the stem the lower leaves are invaded and soon wilt. The leaf petiole in this case remains upright, while the leaflets droop downward, become dry and brown, and persist until they are destroyed by wind or rains. As the disease progresses upward, the remaining leaves, the young shoots, and the immature pods succumb. The upper leaves and shoots of an infected plant are stunted in their development, and become chlorotic before they finally are killed. From this it appears that the pathogene in the xylem vessels produces a toxin which moves ahead of the bacteria and causes an injury in the still uninfected part of the plant. At least the pathogene cannot be isolated from the chlorotic tissue beyond the microscopically visible advancing line of bacteria.

On the leaf

There are two types of symptoms produced on the leaves of beans by *Phyt. medicaginis* var. *phaseolicola*, namely, those due to a systemic infection and those due to a local infection.

The most striking symptom arises from a local infection, and is the spot to which Miss Hedges has applied the term *halo blight*. The spot, which varies in size, may become necrotic, dry, and brown, and appear indistinguishable at the center from other necrotic leaf spots due to the

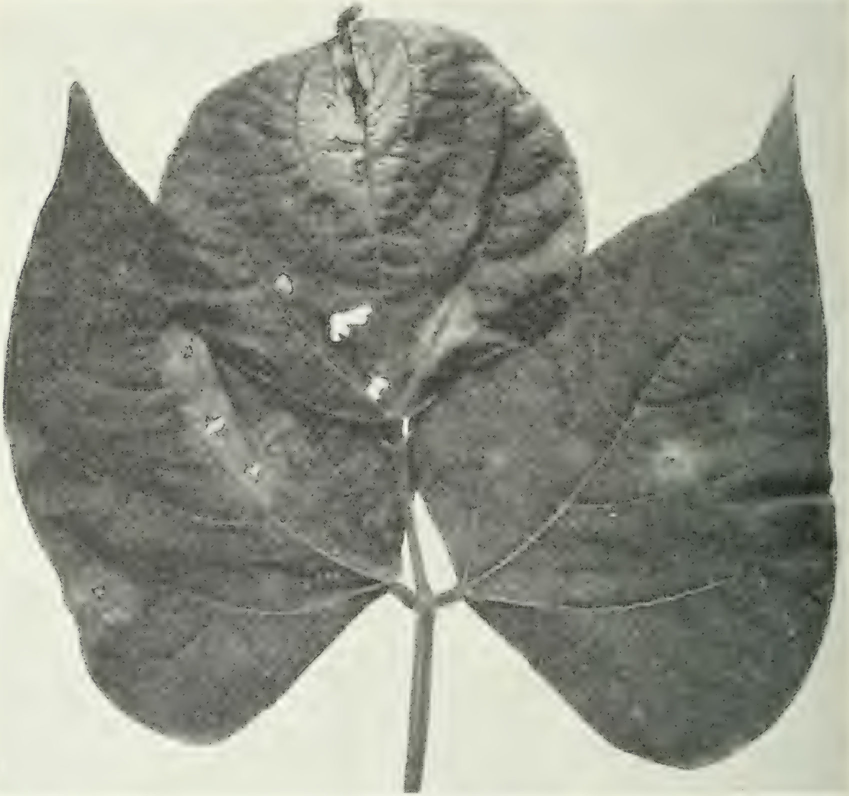


FIGURE 6. THE "HALO" LEAF SPOT OF THE BEAN CAUSED BY PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA

various bacteria described in this memoir. The peculiarity, however, which sets it apart from the leaf spots of the other diseases, is a chlorotic area, or zone, about the dead part. This zone is very pale green, and varies in width from a half inch to an inch. The zone naturally changes with the size of the necrotic area, but a very small spot may show a surprisingly large zone (figure 6). A leaf spot of this type is a fairly good diagnostic symptom, but probably is not infallible. The halo spot is found, as a rule, during cool weather, and later in the season, during the hot weather, it gives place to another type of lesion. The lesions of this latter type are numerous on the leaf, and are small and angular (figure 7).

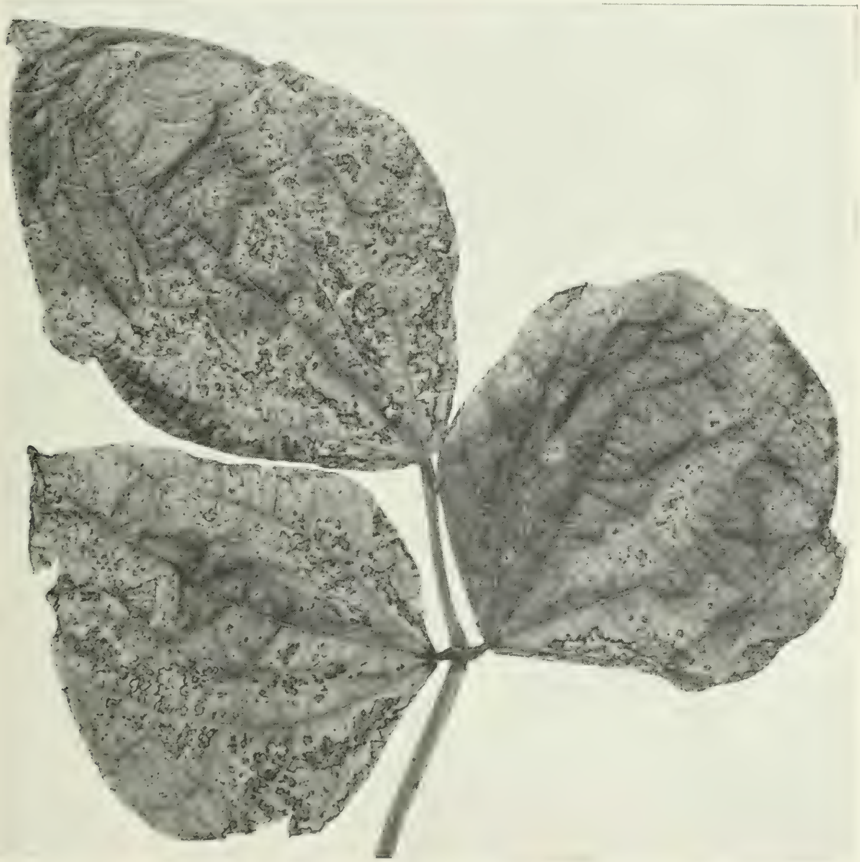


FIGURE 7. A TYPE OF LEAF SPOT OFTEN PRODUCED BY PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA

A chlorotic zone is not so evident, although the lack of a halo is due partly to the fact that the whole leaf is somewhat chlorotic. Nevertheless it is very noticeable that a halo spot is never produced in the hot days of July and August. The angular spots just described probably arise from stomatal infection, and in most cases are an infection of the islets between the veins.

Other necrotic leaf spots due to this organism may arise, similar in

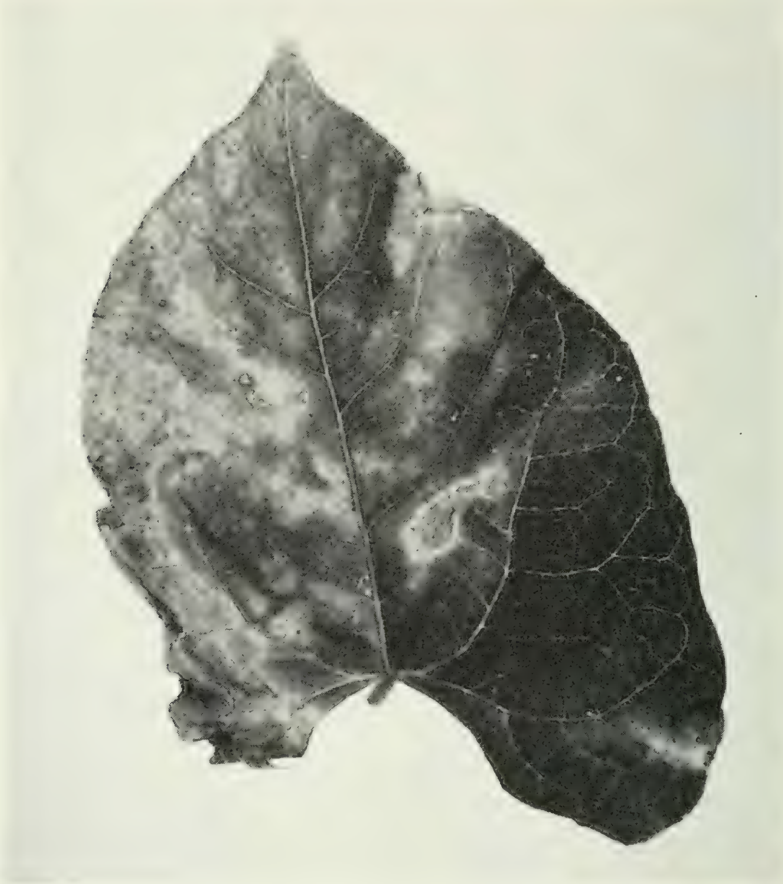


FIGURE 8. PRIMARY LEAF OF A BEAN PLANT WHICH WAS INOCULATED AT THE COTYLEDON NODE WITH PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA

appearance to the ones commonly found in plants infected with *Phyt. phaseoli.* Such spots may vary in size and become fairly large (figure 8). They are not angular, and have no definite, wide, pale green zone although they may be surrounded by a narrow yellowish border. These spots often arise from infections of endogenous origin in systemically infected plants.

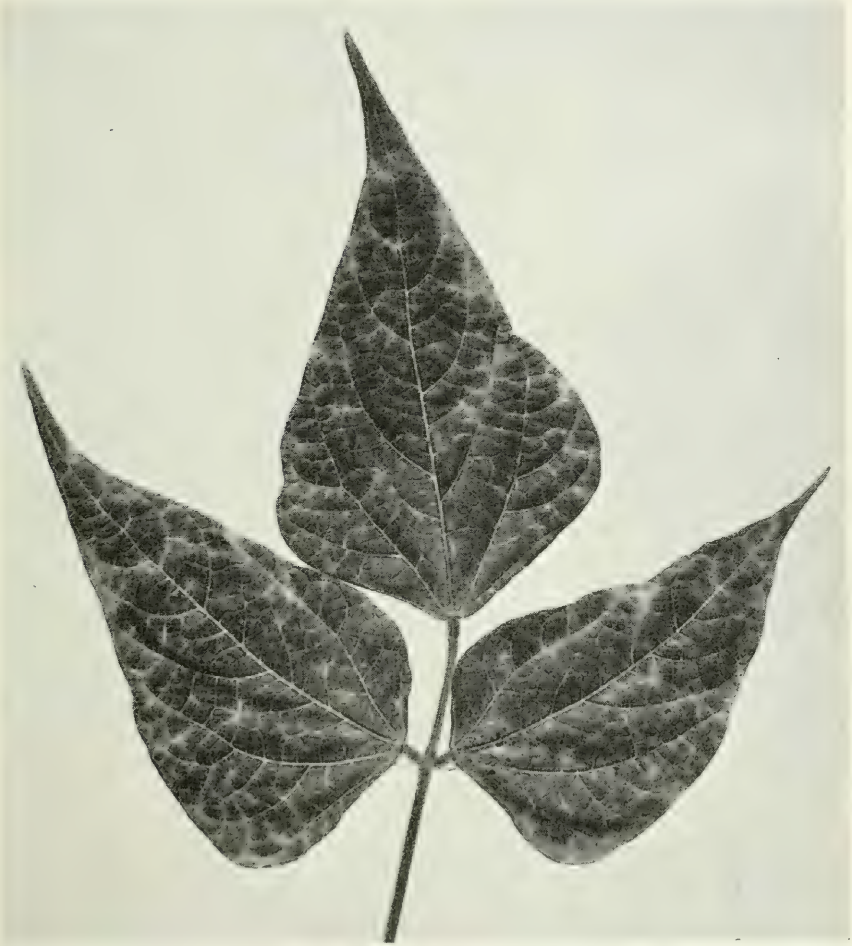


Figure 9. LEAF OF A WHITE MARROW PLANT SHOWING MOTTLING DUE TO PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA IN THE VASCULAR SYSTEM OF THE STEM AND THE PETIOLES

When the infection is local and arises from external infection, the xylem may or may not be involved.

With plants in which the bacteria have multiplied rapidly in the xylem vessels and have become very numerous, certain symptoms appear on the leaves before the pathogene itself has extended to them. There is first a definite mosaic appearance (figure 9), with lighter and darker areas distributed over the leaf, the darker areas being nearer the larger veins. This symptom has been mistaken for the true mosaic, but necrotic lesions appearing on the plant soon make evident the difference. The mosaic-like symptom is not difficult to produce in the greenhouse in artificially inoculated plants.

A more severe manifestation of this type of chlorotic symptom has been noticed frequently in the field on Red Kidney beans. In this case the young leaves of an infected plant are stunted in size, very pale green in color, and extremely crinkled, and the veins are very prominent. Such plants usually succumb very quickly to the disease.

On the stem

Longitudinal streaks due to this pathogene may appear on the stalk, the stem, and the leaf petioles of the bean. On seedlings, the tissue of which is very tender and succulent, such lesions at first are water-soaked and dark green. Later they may dry down and become somewhat reddish, though the color is not always present. On older plants the water-soaked condition is not so evident. Stem lesions, especially those on the seedlings, are local and usually of exogenous origin. When the stem lesions are of endogenous origin a somewhat different picture is produced. The bacteria breaking through the xylem vessels cause longer lesions, often with longitudinal cracks. One very characteristic condition may occur on such lesions which is not found in any of the other bacterial diseases of the bean. The pathogene may ooze out in relatively large droplets on the surface of the stem (figure 10). These droplets are colorless to milky, and in appearance are not unlike those due to *Erwinia amylovora* in fire blight of apples and pears. As a rule they have been observed in the field in the early part of the growing season and during cool, wet weather. They have also been produced in the greenhouse on Red Kidney plants.

On the pods

The individual spots produced by the disease on bean pods are more or less circular in outline, but they may anastomose and give rise to rather irregular lesions (figure 11). These lesions are at first green and water-soaked, but later they may dry out and become sunken and reddish brown in color. In this respect they are similar to spots produced by *Phyt. phaseoli*. In some cases the water-soaked appearance may still be present even after the pods have ripened and dried. Over these pod lesions one

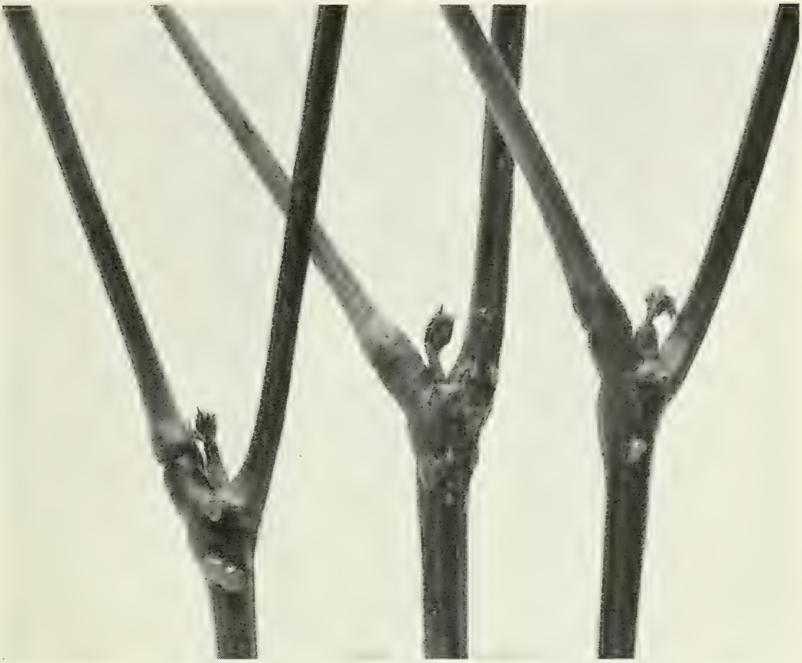


FIGURE 10. OPAQUE DROPS CONTAINING BACTERIA ON THE SURFACE OF BEAN STEMS SYSTEMICALLY INFECTED WITH PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA

may sometimes find a light, silvery crustation due to the bacteria oozing to the surface and drying down. This is a very delicate crust and may be washed away quickly during a rain.

When the bacteria pass through the vascular system of the pedicel and enter the pod, a rather irregular lesion along the suture may be produced (Plate IV, A). Such a suture lesion differs in appearance in no respect from those of the spots as described above, except in position and outline. These lesions are indistinguishable from those produced by *Phyt. phaseoli* and *Phyt. flaccumfaciens.*

On the seeds

This disease may involve the entire seed or only a small part of it. On white seeds the distinguishing characteristic of the spot is the maize yellow to cream color of the affected area. Severely infected seeds are small and wrinkled, and may be completely colored by the pathogene. On colored seeds the spots due to *Phyt. medicaginis* var. *phaseolicola* are

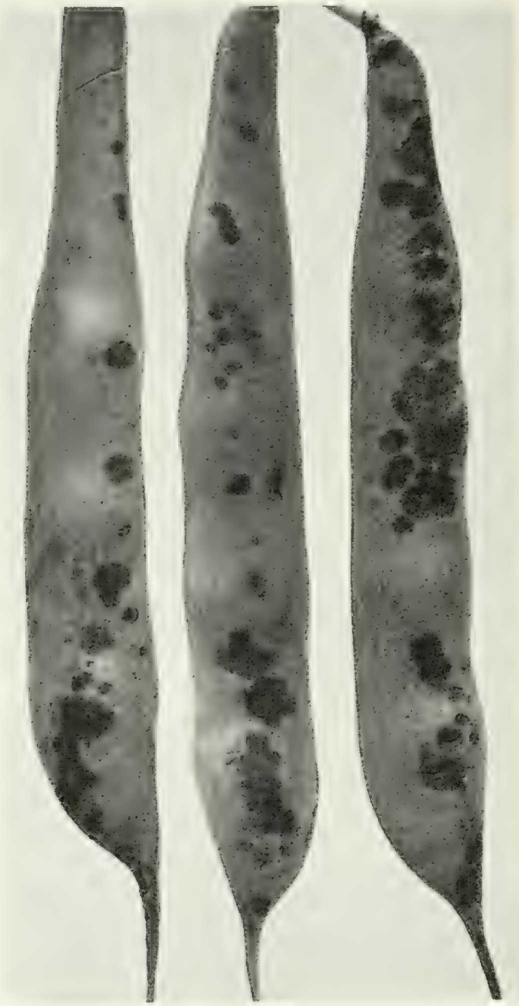


FIGURE 11. PODS OF RED KIDNEY BEAN SHOWING LESIONS CAUSED BY PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA

This photograph would serve equally well to illustrate lesions caused by *Phyt. phaseoli* or *Phyt. phaseoli* var. *fuscans* impossible to differentiate from those caused by the other bacterial or fungous diseases that affect the bean seed.

Phyt. medicaginis var. *phaseolicola* may infect the seed by working its way through the pod tissue from local infections on the outside, or it may enter the seed through the vascular tissue. In the latter case the bacteria spread out underneath the seed coat. Very light infections of either type are of the greatest importance, since they pass unobserved by the pickers in the seed houses and the beans may be used for seed. A few infected plants in a field are all that is needed for the disease to become epiphytotic when the conditions are favorable.

INOCULATION EXPERIMENTS

Many cross inoculations have been conducted with *Phytomonas medicaginis* var. *phaseolicola* on about a dozen legumes and a few non-leguminous plants. In these experiments the organism has shown itself to be rather restricted in its pathogenicity. Besides the common bean (*Phaseolus vulgaris*), only two members of the genus Phaseolus — the scarlet runner bean (*P. coccineus*) and the sieva bean (*P. lunatus*)—proved to be susceptible. All species outside of this genus used in the cross-inoculation experiments were found to be immune. This is a host range more limited than that of *Phyt. flaccumfaciens* or even that of *Phyt. phaseoli.*

Negative results were obtained with the following species of legumes: adzuki bean (*P. angularis*), mung bean (*P. aureus* Roxb.), cowpea (*Vigna sinensis*), yard-long bean (*V. sesquipedalis*), soybean (*Glycine max*), broad bean (*Vicia faba*), hyacinth bean (*Dolichos lablab*), white-flowering lupine (*Lupinus polyphyllus*), kudzu vine (*Pueraria hirsuta*), field pea (*Pisum sativum* var. *arvense*), red clover (*Trifolium pratense* L.), alfalfa (*Medicago sativa*), and sweet clover (*Melilotus alba*). Attempts were made, but without success, to infect the following non-leguminous plants: potato (*Solanum tuberosum* L.), cotton (*Gossypium hirsutum* L.), sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum* Mill.), and tobacco (*Nicotiana tabacum* L.).

ETIOLOGY

Morphology

Phytomonas medicaginis var. *phaseolicola* is a fairly large rod with rounded ends, occurring singly or in pairs. Single cells are sometimes found which are slightly curved, and filaments are not uncommon on certain media. The cells stain well with carbol fuchsin, gentian violet, and Loeffler's methylene blue. Granules always may be demonstrated, and in some cases they are bipolar. On beef-extract agar (pH 6.9), and grown at a temperature of 27° C., single cells of a culture twenty-four hours old measured as follows: 1.9μ (1.35 to 3.6μ) by 1.0μ (0.9 to 1.2μ).

The pathogene is actively motile and a single polar flagellum has been demonstrated. The organism does not form spores, is Gram-negative, is not acid-fast, and is a facultative anaerobe.

Cultural characteristics

(All cultures incubated at 27° C. unless otherwise stated.)

Beef-extract agar slants (pH 7.0). Growth is at first scanty to moderate, filiform, translucent, white or slightly creamy, glistening, smooth except at the edges, which often are wrinkled. The consistency is butyrous, but in old cultures it may be brittle. There is no change in the medium.

Beef-infusion agar slants (pH 7.0). The growth is similar to that on beef-extract agar. A green fluorescent pigment has never been observed on this medium.

Beef-extract agar plates (pH 6.9). Three-days-old colonies are approximately 2 millimeters in diameter, white but bluish by transmitted light, smooth with edges entire. Later, the growth often shows a zonation with edges slightly undulating (Plate VI, E).

Beef-extract bouillon (pH 7.0). The broth becomes cloudy within twenty-four hours. A delicate pellicle appears, which is readily broken up and thrown to the bottom. A white granular sediment is formed.

Gelatin stab (pH 6.6). (Temperature 21°C). In the original description of this organism, it was stated that arborescent growth occurs on this medium and that no liquefaction takes place. Further work with the pathogene on other gelatins, however, showed that this is not altogether correct. Tests with one of the original strains and with several recently isolated strains proved the following to be nearer the truth:

Growth appears along the stab in twenty-four hours; this sometimes becomes somewhat arborescent. At the end of two weeks a slight liquefaction begins, which proceeds very slowly. At the end of six weeks the liquefied gelatin is often like a heavy oil. (The mistake in the original description was probably due to the gelatin used.)

Milk. No separation occurs. At the end of six weeks the milk appears somewhat yellowish brown in color.

Litmus milk. In five days the litmus begins to turn blue, and it remains so throughout the experiment. At the end of six weeks the medium is a rather muddy blue.

Brom cresol purple milk. The milk begins to turn blue in five days, and finally becomes a brilliant bluish purple. There is no further noticeable change.

Uschinsky's solution. There is a slight variation with the different strains. A light clouding occurs in forty-eight to seventy-two hours, sometimes with a delicate surface film. A greenish color appears in three to eight days. Many shades of this color may be noticed, but at the end of six weeks all cultures are a light grape-green. The growth is very cloudy to turbid, with a heavy white pellicle and a heavy white sediment.

Fermi's solution. There is a light cloudy growth in two to three days. The growth is at first white, with a good pellicle. After several weeks a light green color appears, which, however, never becomes distinct. A heavy, stringy, white sediment is formed.

Cohn's solution. There is no growth.

Potato cylinders. A very light colorless growth appears along the streak in approximately twenty-four hours. In some cases the growth is barely visible. The potato cylinders turn somewhat gray.

Cellulose digestion. Fair growth is obtained in the broth, but there is no digestion of cellulose. Three strains were tested, all of which had been in culture for approximately a year.

Starch agar plates (pH 6.6). Plates streaked five or six times, and tested at the end of two, six, and ten days, show no digestion of starch. In determining this, the plates were flooded with a saturated solution of iodine in 50-per-cent alcohol.

Dextrose broth. A cloudy growth occurs in twenty-four hours in the open arm of a fermentation tube. A light acid reaction appears in one to three days, and gradually fades back to the alkaline side. In some cases there is growth in the closed arm of the tube. A pellicle is formed. There is a slight acid production, but no gas.

Saccharose broth. A light cloudy growth occurs in twenty-four hours. A light acid reaction appears in one to two days, and the medium then gradually becomes alkaline. The color reaction is more vivid in test tubes than in fermentation tubes. Growth occurs sometimes, but not always, in the closed arm of a fermentation tube. There is a slight acid production, but no gas.

Lactose broth. A light cloudy growth appears in twenty-four hours. There is no further reaction, and no acid nor gas is produced.

Maltose broth. A light cloudy growth may be noted in twenty-four hours. No acid nor gas is produced.

Indol production. A good cloudy growth appears in trytophane broth in twenty-four hours. Tests made for indol at the end of one and of four days, by the Ehrlich-Böhme method, were negative.

Nitrate reduction. The organism does not reduce nitrates to nitrites when grown on nitrate broth and tested at the end of two, four, and eight days with sulfanilic acid and a-naphthalamine in acetic acid.

Hydrogen-sulfide production. A very light to fair growth is obtained on Wilson's medium, but the reaction is negative. A good growth is obtained in lead-acetate agar. From a negative reaction to a very faint browning occurs along the stab.

Chromogenesis. This organism apparently belongs to the green-fluorescent group. Its ability to produce this pigment, however, is weak. The production of a green color, even by recently isolated strains, has never been noticed on beef-extract agar, bouillon, or gelatin. The pigment is formed, nevertheless, in Uschinsky's solution, and to a less extent in Fermi's solution. The cultural characteristics of this pathogene agree very closely with those of other members of the green-fluorescent group.

Oxygen relations. The oxygen relationship of this organism varies with the strains tested. Certain strains always appear to grow in the closed arms of fermentation tubes in dextrose and in saccharose broth, while other strains vary in this character. In shake agar cultures in which a beef-extract agar with 1 per cent of dextrose was used (pH 7.0), one strain did not produce colonies more than 3 millimeters below the surface, while in other strains colonies were found as deep as 3.5 centimeters below the surface. One strain isolated from bean pods collected in Switzerland had the largest colonies, and the greatest number of them, from 6 to 7 millimeters below the surface.

Longevity. The pathogene, while it frequently does not make a good growth on the various agars, is a hardy organism and does not readily die in pure culture. Several cultures on beef-extract agar kept at a temperature of 3° to 6° C. lived for fifteen months. At the end of that time, subcultures proved to be very virulent when inoculated into bean seedlings.

Thermal death-point. Test tubes of a uniform thickness, and containing 10 cubic centimeters of a beef-extract broth, were used in these experiments. Two lots of broth were used at various times, having, respectively, a pH value of 6.9 and 7.0. These tubes were immersed in a water bath for ten minutes. The water was kept agitated, and accurate temperature readings were made continuously with a certified thermometer. From a number of such experiments it was determined that the thermal death-point was approximately 49° C.

Brief description

Phytomonas medicaginis var. *phaseolicola* is a non-spore-forming rod with rounded ends, occurring singly or in pairs. The cells measure 1.35 to 3.6μ by 0.9 to 1.2μ. Filaments are often produced. The organism is motile by means of a single polar flagellum. It stains readily with carbol fuchsin, gentian violet, and Loeffler's methylene blue, showing granules which often are bipolar. It is Gram-negative, not acid-fast, and a facultative anaerobe.

The colonies on nutrient agar are white to colorless, slow- to moderate-growing, concentrically ringed, with edges slightly undulate; gelatin colonies are slow-growing, circular, raised, and somewhat wrinkled; agar streaks are of moderate growth, colorless to white, flat, glistening, translucent, and butyrous at first but later brittle; in nutrient broth a cloudy growth occurs within twenty-four hours and a pellicle is formed; litmus milk becomes an intense blue, with no peptonization; a small amount of acid is produced in dextrose and in saccharose broth, but none in lactose or in maltose broth; no gas is formed in these sugar broths; nitrates are not reduced to nitrites, nor is gas formed in nitrate broth; gelatin is slightly liquefied; starch is not digested; there is no production of indol; hydrogen sulfide is not produced or but slightly; there is a light yellow-green turbid growth in Uschinsky's solution, a slight trace of green in Fermi's solution, and no growth in Cohn's solution; the thermal death-point is approximately 49° C.

The organism is pathogenic on the common bean (*Phaseolus vulgaris*), the sieva bean (*P. lunatus*), and the scarlet runner bean (*P. coccineus*).

A NEW BACTERIAL DISEASE OF THE BEAN, FOUND IN NEW YORK AND NEW JERSEY

(Caused by *Phytomonas vignae* var. *leguminophila* n. var.)

In August, 1926, specimens of diseased leaves of a garden variety (name unknown) of *Phaseolus vulgaris* were received from Dr. C. Chupp. These had been collected in a field in New Jersey where the disease was said to be very severe. The lesions on the leaves were rather unusual in appearance. They were smaller than those spots which one associates with *Phytomonas phaseoli* or *Phyt. medicaginis* var. *phaseolicola.* They were red-brown in color, and when the specimens were received in Ithaca the lesions appeared to be somewhat raised above the surrounding tissue.

In an attempt to isolate a pathogene from the spots, numerous white bacterial colonies appeared in the plates. They were larger and more rapidly growing than *Phyt. medicaginis* var. *phaseolicola*, and on beef-extract agar they produced a green fluorescent pigment. Inoculation experiments were conducted immediately to determine the pathogenicity of the organism for the bean plant. In these first experiments the incubation period was short; infection appeared in two days when seedlings were inoculated by pricking the stem, and the death of the seedling occurred within a week.

In the following year (August, 1927), specimens of diseased bean leaves were again received which had been collected in the same field in New Jersey. Some of the lesions on these leaves were large and of the type usually considered to be caused by *Phyt. phaseoli.* The majority, however, were small, and were similar to those described above. Nevertheless, in no instance was a green fluorescent organism isolated, and in every attempt at isolation, from no matter what type of lesion, *Phyt. phaseoli* appeared in the plates in abundance.

On September 3, 1926, diseased leaves of the yard-long bean (*Vigna sesquipedalis)* were collected in the vegetable-garden plots on the university farm at Ithaca, New York. The lesions were similar in appearance to lesions caused by *Phyt. phaseoli* on leaves of *P. vulgaris*, but were somewhat lighter in color. *Phyt. phaseoli*, however, is not pathogenic to *V. sesquipedalis.* A white organism producing a green fluorescent pigment was isolated from these lesions, and also was found to be pathogenic to *P. vulgaris.* This strain of the organism, which differed but slightly from that from New Jersey, is referred to in this memoir as the " Q strain."

For use in further study, for inoculation purposes and to determine the cultural characteristics, the two strains of the pathogene were reisolated from artificially inoculated bean plants showing the disease, and were transferred from dilution culture plates.

INOCULATION EXPERIMENTS

On the bean

This disease has not been studied in the field under natural conditions. What is known concerning it has been learned by examination of plants artificially inoculated, and usually under greenhouse conditions. The variety of bean used in the majority of cases was Wells' Red Kidney, a variety which proved to be highly susceptible to the pathogene.

When the organism is introduced into the stem of a bean seedling or a young plant, the disease appears within approximately four days. This is an incubation period which does not differ greatly from those of several of the other bean pathogenes. The mode of attack, however, differs somewhat from that of the other pathogenes with the exception of *Phytomonas viridiflava.* The organism here considered is not a vascular parasite, as is *Phyt. flaccumfaciens,* nor a facultative vascular parasite, as are *Phyt. phaseoli* and *Phyt. medicaginis* var. *phaseolicola.* It does not show a preference for the vascular system, although it sometimes follows the xylem vessels. Consequently, when inoculated into the stem the bacteria seem to invade all tissues. A sunken necrotic lesion ensues, but the epidermis usually remains intact except at the point of inoculation. The plant parts above this lesion die, due to their being cut off from the roots and not necessarily due to the invasion of the organism in these parts. When a seedling wilt results from an artificial inoculation, it is often accompanied by a toppling-over of the plant at the point of inoculation. If not all of the vascular tissue is affected at this point, the plant may recover somewhat.

The disease never appears to be systemic without showing lesions along the stem. Older plants that have become woody are more or less resistant to the pathogene. In such cases the lesion may be on only one side of the stem and the plant will continue to live. In all cases of infection on the stem and the leaves, the organism works rapidly at first, but after a few days the border of the lesion does not extend appreciably. Either the invasive ability of the pathogene diminishes, or a certain resistance is shown in the host. This mode of attack is entirely different from those of *Phyt. phaseoli, Phyt. phaseoli* var. *fuscans, Phyt. flaccumfaciens,* and *Phyt. medicaginis* var. *phaseolicola,* in all of which the persistency of the pathogene remains fairly consistent.

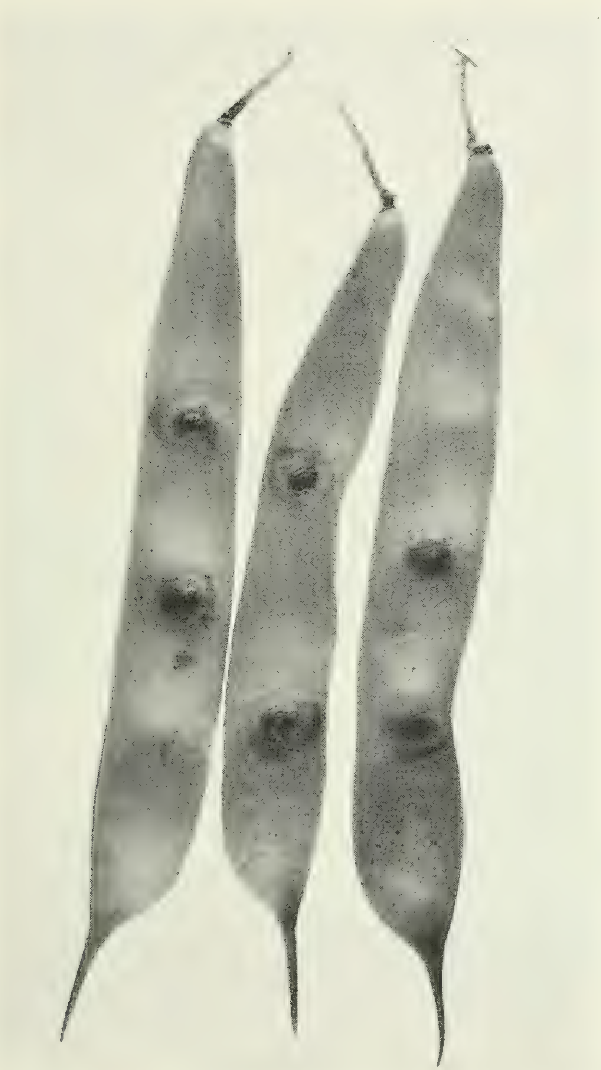


Figure 12. RED KIDNEY BEAN PODS SHOWING RING SPOT LESIONS RESULTING FROM ARTIFICIAL INOCULATIONS WITH PHYTOMONAS VIGNAE VAR. LEGUMINOPHILA

A very characteristic and peculiar lesion occurs on the pods of Wells' Red Kidney. About forty-eight hours after inoculation a ring spot appears, somewhat less than a half inch in diameter and circling the point of inoculation. The color of the ring is at first pale brown, and that of the tissue within is a normal green. This ring spot does not retain its identity long, since the central tissue soon collapses and turns brown. These spots never show a water-soaked condition at any stage, and usually at the end of a week they are dark olive-buff to tawny olive in color.

When bean plants are sprayed with a water suspension of the organism and kept in a moist chamber for twenty-four hours, small spots are produced, but only on the young leaves. These lesions are very small, 3 millimeters in diameter, and under the conditions of observation have never expanded.

The Q strain, besides being a weaker pathogene than the New Jersey strain, never produced the ring spot on the bean pods. Otherwise the symptoms of the two strains are similar.

Cross inoculations

This pathogene, especially the New Jersey strain, has a very wide host range among the leguminous plants. Of twelve species of legumes used in inoculation experiments, eight proved to be susceptible. Experiments were not conducted with any non-leguminous plants. All tests were made in a greenhouse with a fairly constant temperature of approximately 80° F. Inoculations were being made continuously on seedlings of *Phaseolus vulgaris*, to make certain that a virulent culture of the bacterium was being used. When the virulence lagged, reisolations were made.

In all cases, seedlings or young plants of the species being tested were employed. In most cases, inoculations were made also on pods of such species. It was practically impossible, however, to obtain in the greenhouses pods of the scarlet runner bean or of the kudzu vine. The following species proved susceptible to the disease: sieva bean (*P. lunatus*), white-flowering hyacinth bean (*Dolichos lablab*), kudzu vine (*Pueraria hirsuta*), cowpea (*Vigna sinensis*), yard-long bean (*V. sesquipedalis*), soybean (*Glycine max*) variety Wilson, and broad bean (*Vicia faba*). Good infection was obtained on the pods of soybeans, but not on the seedlings.

Negative results occurred when inoculations were made on scarlet runner bean (*P. coccineus*), white-flowering lupine (*Lupinus polyphyllus*), field pea (*Pisum sativum* var. *arvense*), and sweet clover (*Melilotus alba*).

The foregoing results were obtained with the New Jersey strain of this organism. The Q strain caused infection only on the common bean, the kudzu vine, the cowpea, and the yard-long bean. This strain, not only in its cultural reactions but also in its pathogenicity, appeared to be weaker than the other. The type of lesion produced on the above-named plants, however, appeared to be the same. Considering the number of species, and even of genera, that the New Jersey strain infected, it seems strange that the scarlet runner remained immune. Many and repeated inoculations were made on this species, however, without any positive results.

Inoculations were always made on seedlings or young plants of all the species tested. Infections in all cases were similar to those occurring on the Red Kidney seedlings. The disease appeared, and in several days spread rapidly from the point of inoculation, and then further invasion of the tissue ceased altogether. In a few cases, inoculations were made on the pods of some of the above-named species. This was done by pricking the tissue with a sharp-pointed instrument. The following peculiarities were noted: a more-or-less water-soaked lesion occurred on pods of *Phaseolus lunatus*, while a dry red-brown spot appeared on pods of *Vigna sinensis*; the spots on pods of *Glycine max* (Plate IV, D) were irregular in shape, and dark brown to almost black in color. A ring spot was produced in some cases, but not in all, on pods of *Dolichos lablab*.

One attempt was made to obtain infection by spraying seedlings of the yard-long bean, the soybean, and the lima bean, but with no success.

As stated above, infection was obtained in *Vicia faba*. This is the only organism described in this memoir which attacked this species. The plants which were inoculated were from 9 to 12 inches high, but the stems were still succulent. Black, sunken lesions occurred at the point of injury and extended up and down the stem. Leaves adjacent to the spots withered and turned black, and leaves far above them turned black about the tip. Although none of the plants died, they became stunted and very ragged in appearance.

ETIOLOGY

Morphology

The pathogene, when grown on beef-extract agar (pH 6.9), is a fairly large rod with rounded ends, occurring singly or in pairs. In a few cases the rod is slightly curved. A culture twenty-four hours old, grown at 27° C. on the above medium, with a Congo-red negative stain, had the following dimensions: 2.02μ (1.35 to 3.6μ) by 1.07μ (0.6 to 1.8μ).

The organism stains readily with carbol fuchsin and gentian violet. It is Gram-negative and is not acid-fast. It is actively motile, with one to two polar flagella. Capsules have not been demonstrated; Huntoon's method was employed for this test.

Cultural characteristics

(All cultures incubated at 27° C. unless otherwise stated)

Beef-extract agar slants (pH 7.0). A good growth appears along the streak within twenty-four hours, white to almost colorless. The growth on a three-days-old culture is filiform, white, translucent, smooth, and glistening. Freshly isolated cultures produce a marked green fluorescent pigment. The ability to produce this pigment diminishes in strains grown long in culture.

Beef-infusion agar slants (pH 7.0). A very good whitish transparent growth occurs along the streak, similar to that on beef-extract agar. A very marked yellow-green fluorescent pigment is produced.

Beef-extract agar plates (pH 7.0). Seven-days-old colonies are white to hyaline, smooth, amorphous, with edges entire. They are butyrous in consistency, and not more than 5 millimeters in diameter. Q strain appears bluish to opalescent by transmitted light. Older colonies may show a zonation.

Beef-extract bouillon (pH 7.0). The broth becomes cloudy in twenty-four hours, and very cloudy within two days. A light flocculation is produced in seventy-two hours, and within a week a whitish granular sediment is formed.

Gelatin stab (pH 6.6). (Temperature 21° C.) Growth appears along the stab within twenty-four hours, with slight liquefaction at the surface. This liquefaction is at first turnip-shaped, but it proceeds rapidly and soon loses any characteristic shape. Complete liquefaction occurs in about ten days. The liquefied gelatin becomes cloudy, and a heavy sediment forms within it which is slightly yellowish. A green fluorescent pigment is produced. With the Q strain, liquefaction proceeds more slowly and is not complete for six weeks.

Milk. Separation begins on the fifth day, and occurs throughout the culture on the sixth day. Further changes are slow, but at the end of six weeks the culture appears as a muddy yellow liquid. With the Q strain, clearing is very slight.

Litmus milk. A slight blue clearing appears at the top of the culture in two days. This clearing continues, and is nearly complete at the end of eight days. Litmus is reduced from the bottom of the tube upward. In eleven days a muddy purple appearance is evident, and at the end of six weeks the culture is muddy brown. The Q strain is much slower in reaction.

Brom cresol purple milk. Procedure is similar to that in litmus milk, with the exception of the color. A clear and purple zone appears at the top and extends throughout the tube. The color is a reddish purple by transmitted light. At the end of six weeks the culture is a fairly clear wine-colored liquid.

Uschinsky's solution. There is a slight clouding in twenty-four hours, and the culture becomes very cloudy, with a pellicle, in forty-eight hours. In one week a light flocculation appears, and in five weeks the culture is turbid, with a very heavy sediment. At the end of the fourth day a light greenish color may be observed, which deepens and appears in three weeks as an olivine to malachite green. At the end of six weeks the culture is a pale grass green.

The Q strain is white until the tenth day. The color is not likely to become as intense as with the other strain.

Fermi's solution. Slight clouding appears in twenty-four hours, and increases until a good growth is reached in forty-eight hours. A good pellicle is formed by the fourth day. The culture becomes white and milky, and a heavy sediment is formed. No further change occurs in six weeks.

Cohn's solution. There is no growth.

Potato cylinders. At the end of two days the growth is scarcely visible. The medium becomes gray, but there is no further change at the end of seven weeks.

Cellulose digestion. A good growth takes place in broth, but there is no indication of cellulose digestion.

Starch agar plates (pH 6.6). Plates streaked and tested with a saturated solution of iodine in 50-per-cent alcohol at the end of two, six, and ten days show no noticeable digestion.

Dextrose broth. A cloudy growth occurs in the open arm of a fermentation tube in twenty-four hours, and the medium becomes slightly acid. The acidity increases, but by the tenth day a reaction occurs and at the end of sixteen days all cultures appear alkaline. A cloudy growth appears in the closed arm of the fermentation tube. No gas is produced.

Saccharose broth. A light cloudy growth is noticeable in the open arm of a fermentation tube at the end of twenty-four hours. The broth is slightly acid, and reaches its maximum acidity within three days. The acidity is never so pronounced in test tubes as in fermentation tubes, and the broth in the test tubes reverts to the alkaline side very quickly. A cloudy growth occurs in the closed arm. Acid is produced, but no gas.

Lactose broth. A light cloudy growth occurs in the open arm of a fermentation tube in twenty-four hours. No further change in the broth is noted. No acid nor gas is produced.

Maltose broth. A cloudy appearance may be seen in twenty-four hours in the open arm of a fermentation tube, but there is no acid reaction. The culture appears the same at the end of five weeks, except that there is a pale yellow-brown sediment which has formed gradually.

Shake agar culture. In beef-extract agar with 1 per cent of dextrose (pH 7.0), colonies may be observed from 5 to 8 millimeters below the surface at the end of six days. No further colonies appear after that time.

Indol production. The organism produces no indol when grown in trytophane broth. The Ehrlich-Böhme test was used at the end of one and of four days. Positive results were obtained on this medium with *Escherichia coli.*

Nitrate reduction. There is no reduction of nitrates to nitrites. The organism was grown in nitrate broth, and was tested with sulfanilic acid and a-naphthalamine in acetic acid at the end of two, four, and eight days. Positive results were obtained with *Esch. coli.*

Hydrogen-sulfide production. A good growth is obtained on Wilson's medium, but there is no positive reaction. On lead-acetate agar, at the end of a month there is a very slight browning along the stab.

Chromogenesis. The organism produces a water-soluble green fluorescent pigment on various media. This pigment is the most noticeable in Uschinsky's solution; it appears on beef-extract agar and on beef-infusion agar, but not in Fermi's solution. A recently isolated organism produces the pigment better than do the older strains.

Loss of virulence. When the New Jersey strain of this pathogene was first isolated, its virulence was exceedingly high. When it was inoculated into a bean seedling or a young plant, the disease progressed rapidly and the plant was killed in approximately a week. The invasive ability of the pathogene, however, gradually diminished in pure culture, until at the end of eight or nine months it was able to produce only a small lesion on a bean seedling. Its ability to produce a pod spot was not reduced so greatly. In May, 1927, the organism was inoculated into a pod of the sieva bean (*Phaseolus lunatus*). This pod was kept in the laboratory in a dry condition until October of the same year, when the pathogene was reisolated. It was found then that the organism had regained its former virulence. This process was repeated several times during the winter of 1927–28 with the organism, so that its virulence might be retained and it might be used in conducting inoculation experiments.

Thermal death-point. Test tubes of a uniform thinness, and containing approximately 10 cubic centimeters of beef-extract broth (pH 7.1), were used. Two large loops full of a broth culture twenty-four hours old were added to the tubes, and the tubes were immersed in a water bath for ten minutes. The water was kept agitated, and accurate temperature readings were made continually with a certified thermometer graded in tenths of degrees. The thermal death-point appeared to be approximately 49° C.

Nomenclature

It has been pointed out by the writer in a previous article (Burkholder, 1930) that the green fluorescent bacteria which cause plant diseases are a rather closely related group. It is not surprising, then, to find that the pathogene under consideration here agrees so closely in its cultural reactions with one of the nineteen or more previously described species. The species to which it is very closely allied is *Phytomonas vignae*, which also attacks certain leguminous plants. There are several distinct differences between the two pathogenes, however, both in cultural characteristics and in host range, which would keep them from being considered identical. *Phyt. vignae* is described as producing an abundant growth on potato cylinders and causing no change in the color of this medium. In Fermi's solution it produces a green-yellow pigment. The organism dealt with here will barely grow on potato, and causes the medium to turn gray; and in Fermi's solution a white, milky growth is produced. The differences are not great, but they do show that the two pathogenes are not the same.

There is a more pronounced difference in their pathogenicity, however. With *Phyt. vignae*, Gardner and Kendrick (1925) obtained negative results on garden beans, soybeans, and broad beans. Infection was produced in all of these species by the organism under consideration here. So susceptible are the garden bean and also the broad bean, that there could be no mistake concerning this character. Consequently it is felt that this is a distinct pathogene, although one closely related to the cowpea organism. For these reasons it is held as a variety of *Phyt. vignae*, and because of its broad host range is given the name *Phytomonas vignae* var. *leguminophila* n. var.

Brief description

Phytomonas vignae var. *leguminophila* is a non-spore-forming rod with rounded ends, occurring singly or in pairs. The cells measure 1.35 to 3.6μ by 0.6 to 1.8μ. The organism is motile by means of one to two polar flagella. It is Gram-negative, not acid-fast, and a facultative anaerobe.

The colonies on beef-extract agar (pH 7.0) are round, white to hyaline, smooth and amorphous, with the edges entire, producing a water-soluble green fluorescent pigment; nutrient broth is cloudy within twenty-four hours; gelatin liquefaction is fairly rapid; milk turns alkaline, clears, and later becomes muddy; litmus is reduced; a green fluorescent pigment appears in Uschinsky's solution; there is a white milky growth in Fermi's solution; starch is not digested; growth is very slight on potato; acid is produced in dextrose and in saccharose broth, but none in lactose or in maltose broth; gas is not produced in these sugar broths; indol is not produced; nitrates are not reduced to nitrites; hydrogen sulfide is not produced or but slightly; the thermal death-point is approximately 49° C.

The organism is pathogenic on the common bean (*Phaseolus vulgaris*) and on a number of closely related species.

A HITHERTO UNREPORTED BACTERIAL DISEASE OF THE BEAN, FROM EUROPE

(Caused by *Phytomonas viridiflava* n. sp.)

In August, 1927, green-bean pods were observed in a market in Lucerne, Switzerland, showing lesions which, from appearances, were due to a bacterial invasion. The lesions were rather reddish brown in color, and were circular in outline except where anastomosing occurred. Judging from the pod, the bean was of a Refugee green-pod type. A number of these pods were collected and dried, and later, in October of the same year, an attempt was made to isolate an organism. In making these isolations, small parts of the lesions were macerated in sterile water, and dilution plates were poured from these. Bacterial colonies, white to cream yellow in color and producing a green fluorescent pigment, appeared in profusion in all the plates. In the following experiments, three different strains isolated from three different pods were used. The strains were, however, practically identical.

In testing the pathogenicity of these organisms, bean seedlings were inoculated at the cotyledon node by pricking and introducing the bacteria at the same time. Two days after the seedlings were inoculated, infection occurred, and within four days the plants toppled over and were dying. The virulence of the organism was extremely high. In no other case under observation had a pathogene acted with such rapidity. For further work with the pathogene, reisolations were made of the three strains and the pathogenicity of each was again tested.

INOCULATION EXPERIMENTS

On the bean

The disease produced by this pathogene has never been studied nor observed in the field on the living host. After the first specimens were collected in the market of Lucerne, all observations and experiments were conducted on plants artificially inoculated. This was done in a greenhouse under constant temperature control of approximately 80° F. The variety of bean used was Wells' Red Kidney. The striking peculiarities of the disease were observed under these conditions, and they are such as to set it apart from any of the other bacterial blights of the bean.

In general, this Swiss organism appears to be the most virulent of the bacterial pathogenes treated in this memoir. At least, its incubation period is the shortest, and its invasive ability for the first few days is the greatest. When inoculated into a young plant, the organism spreads rapidly and invades all the tissues within its path, killing them and producing a necrotic lesion. Infection appears in about two days. The extent of the invasion, however, is reached possibly by the fifth day or in a little less than a week. Then the invasive ability of the pathogene apparently ceases altogether. Whether this is owing to the organism or is a host reaction has not been determined.

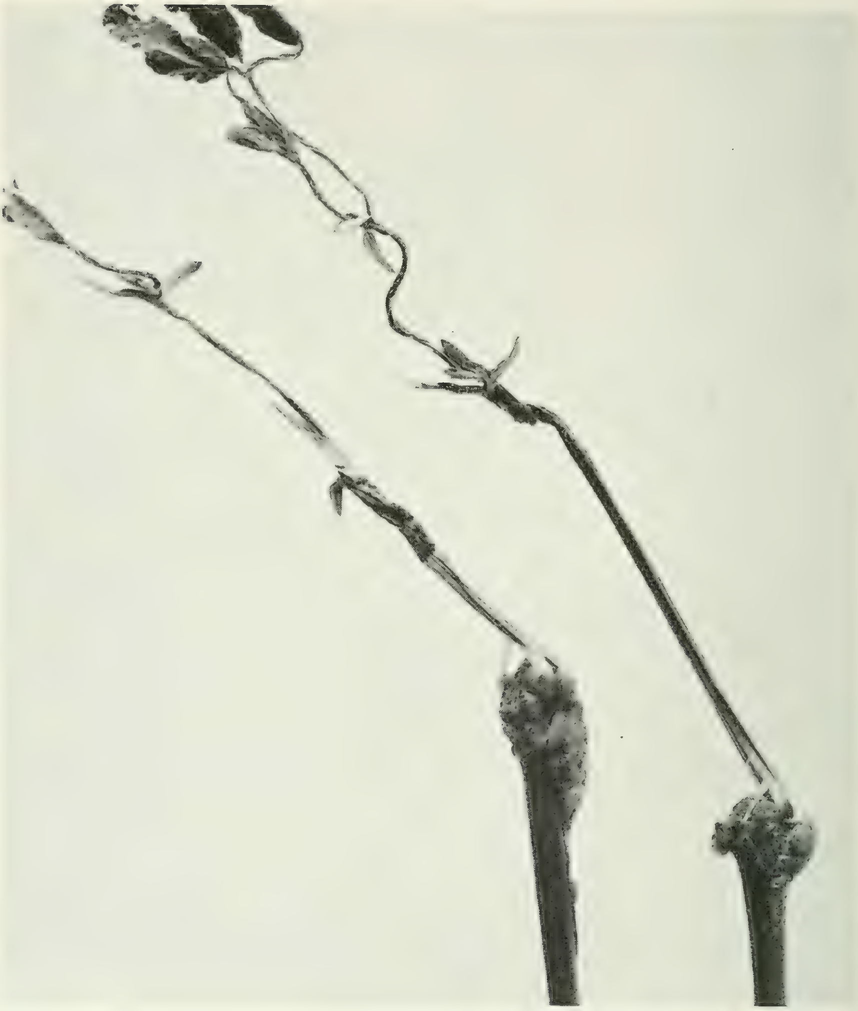
The lesion on the main stem of a young plant will extend probably less than an inch above and below the point of inoculation. This depends to a certain extent on the age and the succulency of the tissues involved. With the killing of the cells throughout the main stem, all parts of the plant above a lesion will die. The collapse in most cases is sudden and complete. This death of the upper part of the plant is in no respect similar to the wilt due to the xylem invasion by many of the other bacterial bean pathogenes.

Below the lesion the tissue of the plant remains healthy, and if a toxin is formed by the bacteria it is not one which rapidly spreads ahead of the invaded cells, as is found in the case of *Phytomonas medicaginis* var. *phaseolicola*. There is no gradation between the dead tissue and the healthy, as may be found in many necrotic diseases; instead, a sharp line of demarcation exists between the two tissues, and this remains stationary since the disease does not move downward. The healthy tissue, however, begins to form a gall, which breaks out from underneath the epidermis and often becomes twice the diameter of the original stem (figure 13).

It is reasonable to expect that the host plant would react by the formation of such a gall merely because of the accumulation of food material at this point. In other words, the reaction may be due to the mechanical injury of the disease. *Phyt. vignae* var. *leguminophila* may cause a similar type of necrotic lesion on the bean plant, but in no case has gall formation been observed. This would appear to indicate that the pathogene under consideration played some part in the formation of the gall. This indication was strengthened further when it was observed that hypertrophy occurred about relatively small lesions caused by the Swiss organism on the stems of sieva beans. It must be admitted, however, that no careful histological studies or attempts have been made to ascertain whether bacteria are present in the galls or whether the bacteria produce a substance which would stimulate gall formation.

The action of the pathogene on the bean pod is somewhat different from its action on the stem. On the pod no hypertrophy has been observed, and, while the bacteria move rapidly in the tissue, there is not that flash of invasion followed by sudden ceasing of growth which is found in the stem tissue. If inoculations are made by injuring the surface of the pod, the pathogene progresses rapidly from the point of inoculation and produces a spot which is slightly sunken and from pecan brown to walnut brown in color. There is no green, water-soaked appearance, as is found in the early stages of pod lesions produced by *Phyt. phaseoli*, its variety *fuscans*, and *Phyt. medicaginis* var. *phaseolicola*, or by the suture lesions produced by *Phyt. flaccumfaciens*. The spot has more the appearance of a late stage of a spot produced by *Phyt. vignae* var. *leguminophila*. In certain instances when the injury to the pod tissue has been deep, the bacteria develop rapidly and ooze out with the sap in small, reddish brown drops.

In a few cases, inoculation experiments were conducted on seedlings and on pods without injuring the plants. The seedlings were sprayed with a suspension of the bacteria in water, and a similar suspension was painted



FIGURES 13. BEAN PLANTS THAT HAVE BEEN INOCULATED WITH PHYTOMONAS VIRIDIFLAVA, SHOWING GALL FORMATION BELOW THE DEAD TISSUES

on the pods with a camel's-hair brush. In no case was infection produced. The experiments were too few in number, however, and were not conducted under sufficiently varied conditions, to permit of drawing any definite conclusions.

Cross inoculations

To determine the host range of this green-fluorescent pathogene, and its pathogenic relationships to others of its type, inoculation experiments were conducted on a dozen species of legumes besides the common bean (*Phaseolus vulgaris*). The experiments were made in a greenhouse at a temperature of approximately 80° F. Seedlings were used in all cases, and in a few instances green pods of certain species were inoculated. Inoculations were made by pricking the plants with a sharp-pointed instrument and introducing the inoculum at the same time.

Good infection was obtained on seedlings of the following species: scarlet runner bean (*P. coccineus*), sieva bean (*P. lunatus*), white-flowering hyacinth bean (*Dolichos lablab*), cowpea (*Vigna sinensis*), and kudzu vine (*Pueraria hirsuta*).

While the results were negative on seedlings of the soybean (*Glycine max*), a high degree of infection was obtained on the pods of this species when these were injured (Plate IV, E). The pod spots were angular in shape but very definite in outline, and were light brown in color with usually a dark border. The variety of soybean used was Wilson. Good pod infection was obtained also on the sieva bean.

Negative results were obtained on seedlings of the following species: white-flowering lupine (*Lupinus polyphyllus*), yard-long bean (*Vigna sesquipedalis*), broad bean (*Vicia faba*), field pea (*Pisum sativum* var. *arvense*), and sweet clover (*Melilotus alba*). Owing to the fact that in culture this pathogene rapidly loses its virulence, and even its pathogenicity, less satisfaction is felt with this list of plants giving negative results than with any other list in the memoir. It is true that more than one set of inoculations were made on the species listed above, and in all sets of experiments certain species in the susceptible list succumbed, showing that the culture used was composed of virulent organisms. Nevertheless, because of the inconsistency with which the pathogene sometimes refused to infect susceptible plants, the negative results were regarded with some uncertainty.

ETIOLOGY

Morphology

From a culture twenty-four hours old on beef-extract agar (pH 6.9), grown at a temperature of 27° C., individual cells are large rods with rounded ends. Considerable variation, however, occurs among them. They measure 2.13μ (1.35 to 3.6μ) by 0.81μ (0.6 to 1.2μ).

The organism stains well with carbol fuchsin and gentian violet. Granules, both central and polar, have been observed. The pathogene is Gram-negative and not acid-fast. Capsules have not been demonstrated. All cultures on agar showed an actively motile organism, which, when stained with Loeffler's flagella stain, was found to have one to two polar flagella.

Cultural characteristics

(All cultures incubated at 27° C. unless otherwise stated)

Beef-extract agar slants (pH 7.0). Growth appears along the streak within twenty-four hours. At the end of three days there is a very good growth, filiform, smooth, glistening, and translucent. The bacteria become cream-colored to definite yellow with age. Cultures are butyrous in consistency. A slight green fluorescent pigment is dissolved in the medium.

Beef-infusion agar slants (pH 7.0). A very good growth occurs, creamy to yellow but otherwise similar in appearance to the growth on beef-extract agar. A very marked yellowish green fluorescent pigment is produced, which is dissolved in the medium.

Beef-extract agar plates (pH 7.0). At the end of a week the colonies are approximately 5 to 7 millimeters in diameter, smooth, naples yellow to cream color, with edges undulating. A weak green fluorescent pigment is dissolved in the medium.

Beef-extract bouillon (pH 7.0). The medium becomes cloudy in twenty-four hours, and very cloudy in forty-eight hours. A light flocculation occurs throughout the tube, and in some cases a delicate pellicle is formed. A sediment, white and stringy, gradually accumulates.

Gelatin stab (pH 6.6). (Temperature 21° C.) Growth appears along the stab within twenty-four hours, with slight liquefaction at the surface. Liquefaction proceeds rapidly, being complete in seven to ten days. It is turnip-shaped at first, and later stratiform. A heavy white sediment is formed, and the liquefied medium becomes cloudy at the surface.

Milk. Clearing of the milk begins at the surface on the fourth to the sixth day, and extends throughout the tube by the end of a week. With age the medium becomes a brownish, muddy liquid, with a slight sediment.

Litmus milk. At the end of three days a clearing begins at the surface, and this gradually extends throughout the medium. Litmus turns slightly blue and is reduced. The milk then becomes muddy, often with a purple tinge.

Brom cresol purple milk. On the third day the medium has become purple, and a light clearing has begun at the surface of the milk which later extends throughout the tube. Within a week the milk is a clear red-purple liquid by transmitted light. At the end of six weeks little further change has occurred.

Uschinsky's solution. The solution is slightly cloudy in twenty-four hours, and very cloudy in several days. A green fluorescent pigment is formed, which is very pale at first and changes through various shades of green until at the end of a week it is an olive-yellow. An orange-yellow sediment is formed.

Fermi's solution. A slight clouding appears in twenty-four hours, and in four days there is a very good growth. In a week a pellicle appears which in some cases becomes very heavy and falls to the bottom of the tube. A green fluorescent pigment appears after several days to a week, and at the end of six weeks this is a water green in color.

Cohn's solution. There is no growth.

Potato cylinders. At the end of two days there is a very slight growth on the surface of the potato, but no further signs are noticeable. The medium turns slightly gray.

Cellulose digestion. The organism grows well in the broth, but there is no indication that the cellulose is attacked.

Starch agar plates (pH 6.6). Plates streaked five or six times, and tested two, six, and ten days after inoculation with a saturated solution of iodine in 50-per-cent alcohol, show no digestion of starch.

Dextrose broth. The broth in the open arm of a fermentation tube becomes cloudy in twenty-four hours. It is at this time slightly acid, and within four days it is distinctly acid, after which it gradually returns to the alkaline side. A yellow-orange to brown pigment is formed in this medium, which is very marked in one strain. In most cases there appears to be a fair growth in the closed arm of the fermentation tube. No gas is produced.

Saccharose broth. A light clouding appears in twenty-four hours in the open arm of a fermentation tube. Growth is better on the second day, but there is no growth in the closed arm of the tube. No acid nor gas is produced.

Lactose broth. A light cloudy growth appears in twenty-four hours and reaches its optimum in four days. No acid nor gas is produced.

Maltose broth. A cloudy growth appears in twenty-four hours. No acid nor gas is produced.

Shake agar culture. In beef-extract agar with 1 per cent of glucose (pH 7.0), colonies are not found lower than 2 millimeters below the surface.

Indol production. The bacteria grow well in trytophane broth, and tests for indol at the end of one and of four days with the Ehrlich-Böhme method have been negative.

Nitrate reduction. Good growth occurs in nitrate broth, and tests for nitrites made at the end of two, four, and eight days with sulfanilic acid and a-napthalamine in acetic acid show no presence of nitrites. A positive test was obtained on the above medium with *Escherichia coli.*

Hydrogen-sulfide production. Growth appears on Wilson's medium within three or four days, but there is no positive reaction. On lead-acetate agar there is a faint browning along the stab. The bacterial mass at the surface appears rather blue-green in color.

Chromogenesis. Several kinds of pigments are produced by this organism on various media. A green fluorescent pigment, which is water-soluble, is formed when the pathogene is grown on beef-extract or beef-infusion agar, and is very conspicuous in Fermi's and in Uschinsky's solution. Also, on the agars the bacteria cells may be of a creamy color in young cultures to a naples yellow in older ones, and in many solutions and broths a yellowish sediment is formed. In dextrose broth an orange-yellow pigment colors the entire medium, and one strain of the pathogene produced it to such an extent that it was difficult to detect the Andrade's indicator in the medium.

Virulence. A loss of virulence, and even of pathogenicity, of the organism occurs in pure culture. This was first noted when a strain isolated in October, and re-isolated on November 10 of the same year, showed little invasive ability on the 1st of January. For making inoculation experiments, and especially cross inoculations, re-isolations were made about once a month. In this way the virulence of the organism was kept from waning.

Thermal death-point. Test tubes of a uniform thickness, and containing 10 cubic centimeters of beef-extract broth (pH 7.1), were used in this experiment. To these tubes were transferred two large loops full of a broth culture twenty-four hours old, and the tubes were immersed in a water bath for ten minutes. The water was kept agitated and accurate temperature readings were made continuously. The thermal death-point appeared to vary somewhat with the strain used, from 48° to 50° C.

Nomenclature

The pathogene under consideration belongs to that group of bacteria which produces a water-soluble green fluorescent pigment, and consequently it is more or less related to all the species of that group. As far as known, however, its primary characteristics do not entirely agree with those of any of the previously described forms. For this reason, and from the fact that the disease which it produces on the common bean (*Phaseolus vulgaris*) is very characteristic, it is here considered as a new species and the name *Phytomonas viridiflava* n. sp. is given to it.

Brief description

Phytomonas viridiflava is a non-spore-forming rod with rounded ends, occurring singly or in pairs. The average size of the cells is 2.13μ by 0.81μ. The organism is motile by means of one to two polar flagella. It is Gram-negative, not acid-fast, and a facultative anaerobe. Capsules have not been demonstrated.

Growth on beef-extract agar streaks is good to abundant, filiform, smooth, glistening, and translucent; the bacteria become naples yellow with age; a green fluorescent pigment is formed on agar and on various other media; bouillon becomes cloudy, with a light flocculation; liquefaction of gelatin is rapid; litmus milk is cleared, and the litmus turns slightly blue and is reduced; a green fluorescent growth appears in Uschinsky's and in Fermi's solutions; there is no growth in Cohn's solution; growth is slight on potato; there is no digestion of starch in starch agar; acid is produced in dextrose broth, but not in saccharose, lactose, or maltose broth; no gas is produced in any of these sugar broths; indol is not produced; nitrates are not reduced to nitrites; hydrogen-sulfide production, if any, is slight; the thermal death-point varies from 48° to 50° C.

The organism is pathogenic on the bean (*Phaseolus vulgaris*) and on a number of related leguminous plants.

ANOTHER STRAIN OF PHYTOMONAS VIRIDIFLAVA

Another strain of *Phytomonas viridiflava* was obtained from diseased specimens of bean leaves collected in England. This strain varied in a few respects from the one obtained in Switzerland, but the difference consisted primarily in the English strain being a weaker parasite. Since it has been shown that the Swiss organism loses its virulence, and even its pathogenicity, in pure culture, virulence is scarcely a characteristic to be considered seriously in this species, especially taxonomically. Certain interesting points of pathogenicity, however, are considered worthy of reporting.

The diseased leaves from which the pathogene was isolated were of the scarlet runner type, and the spots observed on them were, in most cases, small. The largest and most conspicuous of these lesions are shown in figure 14. The leaves were collected in a garden near Canterbury, England, in September of 1927, and the isolation of the pathogene was made a month later. The cultural characteristics and the physiological reactions of the English strain were identical with those of the Swiss strain. This was a sufficient reason for considering them of the same species.

The peculiarities of the English strain of *Phyt. viridiflava* became noticeable, however, when attempts were made to infect with it the bean and related species. When inoculations were made on the seedlings of bean plants, infection was not obtained even though the stems of the plants were injured. The bacteria were unable to establish themselves in the tender young tissue of the growing stem, and the mechanical injury made on the plant quickly healed. Small lesions, nevertheless, were produced on the leaves of several species of plants, including *Phaseolus vulgaris* and *P. coccineus*. In these cases the leaves had been punctured with a needle at the time when the inoculum was applied. The spots always remained small, but on miscropical examination it was found that the bacteria were in abundance in the dead and dying cells. This showed that the organism was pathogenic, but its virulence appeared to be low.

Inoculation experiments were then made on the pods of the common bean and a number of related species. Here the results were different, since this tissue proved to be more susceptible to the pathogene. The

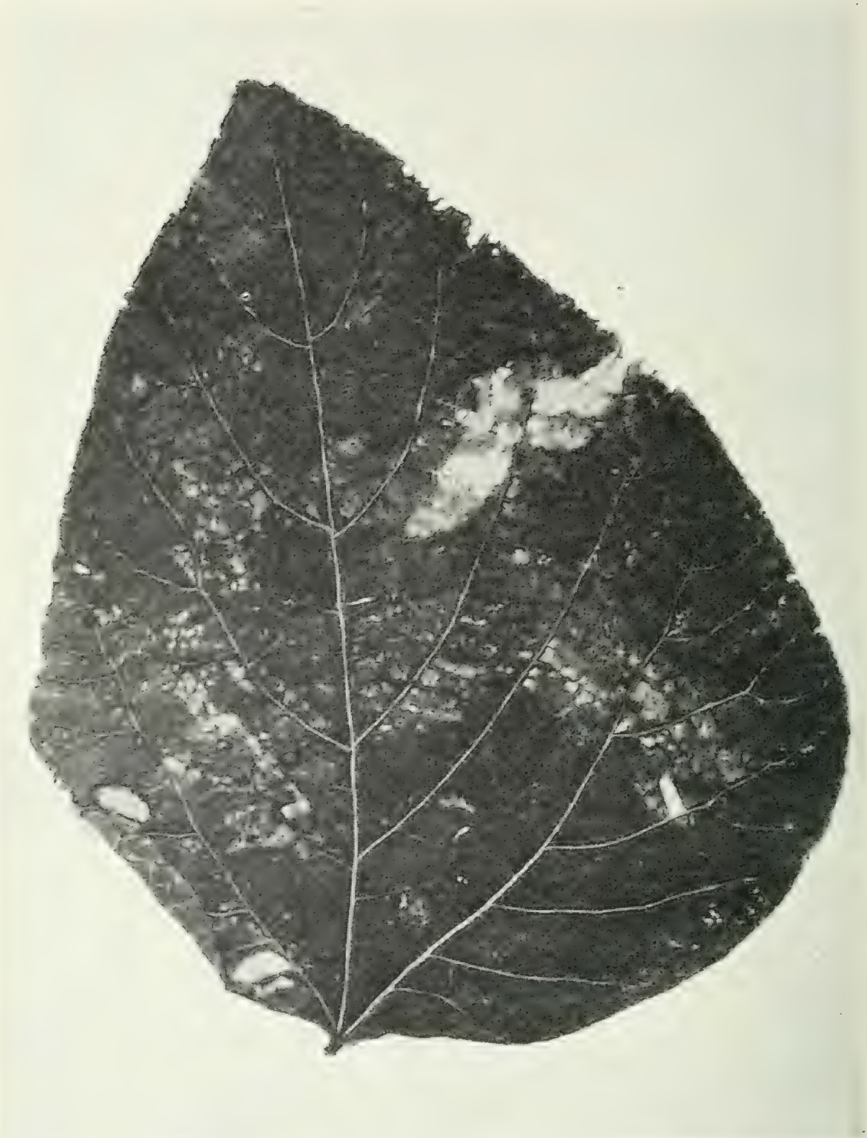


FIGURE 14. LEAFLET OF A SCARLET RUNNER BEAN PLANT FROM WHICH THE ENGLISH STRAIN OF PHYTOMONAS VIRIDIFLAVA WAS ISOLATED

spots that developed on the inoculated pods were identical with those produced by the Swiss strain, and the host range was similar. Pods of all the species listed in cross inoculations with *Phyt. viridiflava* were used in inoculation experiments with the English strain, with the exception of *Vicia faba* and *Pueraria hirsuta*. These species did not set pods under the conditions maintained in the greenhouse.

In conducting inoculation experiments on pods of various species, a further peculiarity of the organism and its relation to the host was observed. When very young pods were used in the experiments, no infection was obtained; but extensive infection occurred on maturing pods. When such pathogenes as *Phyt. phaseoli* and *Phyt. medicaginis* var. *phaseolicola* are used in inoculation experiments, almost the reverse of this is true, and it is the case also with the fungous pathogenes of the bean. The susceptible stage in the pod cannot be coupled with a dying of the tissue, since the pod is susceptible for a considerable time before it shows signs of ripening and while it is still in a green and apparently healthy condition. The very young, rapidly growing pods, however, are resistant to the pathogene. This phenomenon is demonstrated readily on such species as *Phaseolus coccineus* and *Vigna sinensis*. It probably is found also with other species, but it was with these two legumes that repeated inoculations and observations were made on young and on maturing pods.

This host relationship probably is tied up with the low virulence of the pathogene, and a somewhat similar case might be cited here. It has been observed with *Phyt. phaseoli* and *Phyt. vignae* var. *leguminophila* that when grown for some time in pure culture they lose their ability to infect the stem of young plants, to produce either a vascular infection or a definite lesion, as is the custom with each organism. Nevertheless, they still retain their ability to infect bean pods. Experiments have never been conducted, however, to determine their relative invasive ability on the pods in various stages of maturity.

DIFFERENTIATION OF THE BACTERIAL DISEASES OF THE BEAN

The differentiation of the various bacterial diseases of the bean is of no consequence to the average grower. The injuries caused by each disease are somewhat similar in appearance, and the sum total is of considerable economic importance. That much the grower appreciates, and a simple method with which to combat these diseases is all that he asks. For the plant pathologist, however, the ability to separate the diseases may be of considerable importance. Aside from the investigation of the diseases with a view to their control, the desirability of determining their distribution and the severity of their epiphytotics in various localities is well understood. In endeavoring to distinguish the various diseases, the determination of the etiological agent is always the final issue. Nevertheless, with these diseases the isolation of the bacteria and their identification in pure culture is not always possible, and in the majority of cases is not practicable. Too much time and labor would be consumed. If the diseases could be distinguished one from another in the field on a symptom basis, the problem would be greatly simplified. The extent to which the symptoms could be used with certainty should be carefully examined and set forth, together with their limitations. This is attempted in the following discussion.

It should be taken into consideration also that two or more of these diseases may appear on the same plant. In New York, *Phytomonas phaseoli*, *Phyt. flaccumfaciens*, and *Phyt. medicaginis* var. *phaseolicola* sometimes intermingle in the field, and various combinations of these organisms have been isolated from the same diseased individual. Under such conditions a complication of symptoms is set up which is difficult to untangle. Such cases, however, are more likely to be individual, and do not often occur in a bean field.

SYMPTOM BASIS

In the foregoing descriptions of the various bacterial diseases, the most striking and characteristic symptoms of each disease have been pointed out and discussed. In the main these symptoms have been observed in the field on the Red Kidney variety and have been produced on this variety in the greenhouse for purposes of study. To a less degree they have been observed on such common bean varieties as White Marrow, Pea, Refugee, and a few other types found growing in the fields and gardens of New York. More than a hundred varieties of *Phaseolus vulgaris* may be obtained on the market which differ greatly in many of their characters from the Red Kidney bean or the other varieties mentioned above. It is not inconceivable, then, that the symptoms of the bacterial diseases may vary considerably with the variety of beans affected. For this reason it is considered best not to lay too much emphasis on individual symptoms on unknown varieties when endeavoring to separate these bacterial diseases in the field. The Red Kidney and Marrow beans as they occur in New York are the types that are under discussion here.

In considering these symptoms for use as diagnostic characters, certain limitations should be noted. The chief of these limitations arises through the fact that a pathogene may not always produce the same type of symptoms on the bean plant. The disease-producing organism may cause a distinct type of lesion on the leaf, the pod, and other plant parts, but this will not hold in 100 per cent of the cases. Certain anomalies at times are found to exist. These anomalies are seldom to be met with in the greenhouse, where conditions are fairly well controlled, but are found more often in the field, where the environmental factors surrounding the life of a diseased plant can only be guessed at. The causes of these variations in types of lesions may be due primarily to ecological factors. Temperature and moisture, in some instances, appear to play an important part. In other cases the origin of the lesion, whether exogenous or endogenous, may be of significance. The possibility of there being races of the pathogene with different symptom-producing abilities should not be ruled out of consideration, although such cases have not been proved.

Certain general aspects of the diseases which might prove to be of some diagnostic value should be considered first. As already stated, the diseases caused by *Phytomonas phaseoli* and *Phyt. flaccumfaciens* appear to be favored by hot weather, while the amount of rainfall apparently is not an important factor. On the other hand, *Phyt. medicaginis* var. *phaseolicola* occurs with greater severity in cool seasons. This disease at such times may spread rapidly through a field and work with great speed in the individual plant. In New York no other bacterial disease has caused so much damage, and by the very severity of its action one may almost determine the causal organism.

In considering the individual symptoms of the diseases on the plant as of diagnostic value, they are merely pointed out in this discussion, since they have been described in some detail earlier in the memoir. The most noticeable symptoms of these diseases occur on the leaf, and it is here that most of the peculiarities and characteristic lesions are to be found. The halo-blight leaf spot (figure 6) caused by *Phyt. medicaginis* var. *phaseolicola* is a symptom which can scarcely be mistaken or confused with other leaf spots. This organism, however, does not always produce this type of lesion, and in New York, during the hot months, the common symptom is the small angular spot which occurs in such great numbers over the leaf (figure 7). *Phyt. phaseoli*, however, has been known to produce a spot similar to this latter one, and so this organism should always be kept in mind. In plants systemically infected, certain leaf symptoms indicate when *Phyt. medicaginis* var. *phaseolicola* is the causative agent. These are the mosaic appearance of the upper leaves, and, in severe cases, the dwarfed and yellowish crinkled condition of these leaves. However, in the absence of these symptoms, even though the vascular tissue is affected it is not safe to assume that the disease is caused by some other vascular-invading organism. It should be mentioned here, too, that the mosaic symptoms on the leaves have been produced in the greenhouse on Red Kidney beans artificially inoculated with *Phyt. flaccumfaciens*. While this experiment has been repeated a number of times in the greenhouse, the symptom has never been observed in the field due to this latter organism.

Phyt. phaseoli produces, on the leaf, brown necrotic lesions of varying sizes, with yellow borders (figure 1). These spots may enlarge and cover most of the leaf tissue. The lesion illustrated in figure 8, however, caused by *Phyt. medicaginis* var. *phaseolicola*, is not unlike this type of leaf spot.

Infected leaves of beans have been received from Bermuda, and also from New Jersey, with small spots, reddish brown in appearance and somewhat raised above the surface of healthy tissue (Plate II). Pure cultures of *Phyt. phaseoli* only were isolated from such lesions; nevertheless, *Phyt. vignae* var. *leguminophila* also produces this type of lesion on bean leaves, and so it can scarcely be considered a good diagnostic character.

Phyt. flaccumfaciens, as a rule, wilts the entire leaf or leaflet of the bean, but in some instances it has been known to wilt only a part of the leaf, as in figure 5, which shows a rather large necrotic spot. A similar condition may be produced by *Phyt. phaseoli*, if the necrotic area is of endogenous origin.

On the pod, *Phyt. viridiflava* and *Phyt. vignae* var. *leguminophila* produce spots which from the beginning of their formation are brownish and often dry and necrotic. The other bacteria produce spots which are at first deep green and water-soaked in appearance. The water-soaked condition of the lesion may persist, or under certain circumstances the spots may dry down to reddish sunken areas. Over the pod spots produced by *Phyt. phaseoli* a yellowish crustation may sometimes be observed, while a silvery crustation may be produced by *Phyt. medicaginis* var. *phaseolicola*. This crustation is very delicate and is readily washed off by rains, and therefore it is of very little use in differentiating the two diseases. *Phyt. flaccumfaciens*, as has been pointed out, produces on the green pod only water-soaked lesions along the suture, which are of endogenous origin. This symptom, however, can be duplicated by *Phyt. phaseoli*, *Phyt. phaseoli* var. *fuscans*, and *Phyt. medicaginis* var. *phaseolicola*, and consequently it is not a character to be used without discrimination.

On the stem, *Phyt. phaseoli* produces reddish-colored lesions which extend longitudinally with the stem. A stem girdling at the nodes also is produced, and probably in the majority of cases this arises from an infected xylem. *Phyt. flaccumfaciens* produces only the stem girdle. A very striking peculiarity of *Phyt. medicaginis* var. *phaseolicola* is the formation of the bacterial ooze, or exudate, on the stem lesions (figure 10). No other bacterial disease of the bean has been observed to do this, and therefore this symptom should be looked for in endeavoring to determine the disease under observation. When *Phyt. viridiflava* is inoculated into the stem of a growing bean plant, gall formation, as a rule, occurs. This differentiates the lesion from those due to *Phyt. vignae* var. *leguminophila*.

When a systemic infection occurs in a plant, and a wilt results with no definite stem, leaf, or pod lesions, the indications are that the invasive organism is *Phyt. flaccumfaciens*. This pathogene is almost an obligative vascular parasite, and when it is found in other tissues, as in the pod lesion along the suture, it appears to be more of an overflow from the xylem tissue. While *Phyt. phaseoli*, *Phyt. medicaginis* var. *phaseolicola*, and to a less extent *Phyt. phaseoli* var. *fuscans*, prefer the xylem and consequently also cause a wilt, these pathogenes are in no way limited to this tissue. They may cause infection in almost any part of the plant. On the other hand, *Phyt. viridiflava*, and to a less extent *Phyt. vignae* var. *leguminophila*, appear to show no preference for the vascular tissue. When they cause a wilt it is due to the invasion of most of the tissues of the stem, including the vascular tissue, and wilting and death of the plant parts above occur as a natural sequence.

In summing up the information, one can only conclude that the best and surest way to diagnose one of these bacterial diseases in the field is to consider the sum total of all the symptoms displayed, and arrive at a decision from them. Even then one cannot always be correct. In table 1, descriptions of the various characteristic symptoms of these diseases have been brought together and arranged to facilitate diagnosis.

TABLE 1. Major Symptoms Produced by Six Different Bacterial Pathogenes on the Bean (Phaseolus vulgaris)

Pathogene	Wilt	Stem lesions	Leaf lesions	Pod lesions
Phytomonas phaseoli	Result of a vascular invasion	Reddish lesions longitudinal with stem, also stem girdle	Definite necrotic lesions of varying size with yellowish border, Several other types of lesions	Lesions at first water-soaked, angular, anastomosing, drying into reddish sunken areas; lesions along suture of endogenous origin
Phyt. phaseoli var. *fuscans*	Same as with *Phyt. phaseoli*	Same as with *Phyt. phaseoli*, but lesions are inclined to split stem	Same as with *Phyt. phaseoli*	Same as with *Phyt. phaseoli*
Phyt. flaccumfaciens	Result of a vascular invasion. A major symptom	Stem girdle	A part of the leaf, but more often the entire leaf, may wilt	Along suture only, and of endogenous origin; lesions at first water-soaked and green
Phyt. medicaginis var. *phaseolicola*	Result of a vascular invasion	Lesions longitudinal with stem; drops of bacterial exudate sometimes appear	Definite necrotic lesions with light-colored halo; small angular spots; mosaic and yellow symptoms due to systemic infection	Similar to those produced by *Phyt. phaseoli*; a silvery crustation sometimes appears over lesion
Phyt. vignae var. *leguminophila*	Necrotic lesions killing stem, resulting in wilting and death of parts above	Sunken longitudinal lesion which involves all stem tissue	Definite, reddish brown, rather small lesions; wilted parts above stem lesions	Brownish, dry lesions, never water-soaked; sometimes a ring spot
Phyt. viridiflava . .	Definite necrotic lesion killing stem, resulting in wilting and death of parts above	Definite lesions involving all tissue; gall production	(?)	Reddish brown spots, never water-soaked

ETIOLOGICAL BASIS

When it is desirable to be very positive concerning the etiological agent of a bacterial disease of the bean, the isolation and determination of the pathogene is essential. This is not a difficult task, since in certain media the bacteria show considerable differences. Little need be said here as to the technic of isolations. All the organisms grow well on beef extract agar or on potato agar. In the lesions on the plants, the bacteria may be found in abundance and in most instances apparently inhibiting all secondary invaders. At least, by following the ordinary precautions only a few contaminating colonies will appear on the plates. Dilution cultures are usually more satisfactory than tissue plantings.

The morphology of the respective pathogenes is not sufficiently characteristic to be of much help in diagnostic work. One must rely on the cultural reactions. In table 2 a few reactions are given which will sufficiently differentiate all of the organisms. The complete descriptions on preceding pages of the memoir should be consulted also. The color characters may be a little difficult to use, with the exception of the deep

TABLE 2. MAJOR DIFFERENCES IN THE CULTURAL REACTIONS OF SIX DIFFERENT BACTERIAL PATHOGENES OF THE BEAN (PHASEOLUS VULGARIS)

Pathogene	Pigment on beef-extract agar	Gram stain	Sugar fermentation			Litmus milk	Potato cylinders	Fermi's solution
			Dextrose	Saccharose	Lactose			
Phytomonas phaseoli	Bacterial cells a distinct yellow	–	–	–	–	Alkaline, clearing; formation of tyrosine crystals in abundance	Bacteria and potato cylinders become a slimy mass	No growth (?)
Phyt. phaseoli var. *fuscans*	Bacterial cells a distinct yellow; medium becomes brown	–	–	–	–	Alkaline, clearing; liquid turns brown	Bacteria and potato cylinders become a brown, slimy mass	No growth (?)
Phyt. flaccumfaciens	Bacterial cells pale yellow	+	Acid	Acid	Acid	Acid, soft curd	None to fair growth; no change in medium	No growth (?)
Phyt. medicaginis var. *phaseolicola*	Bacterial cells transparent to white	–	Acid slight	Acid slight	–	Alkaline, no clearing	Scant growth	Very pale green fluorescent growth
Phyt. vignae var. *leguminophila*	Bacterial cells transparent to white; water-soluble green fluorescent pigment	–	Acid	Acid	–	Alkaline, rapid clearing	Scant growth	Milky white growth
Phyt. viridiflava	Bacterial cells creamy to yellow; water-soluble green fluorescent pigment	–	Acid	–	–	Alkaline, rapid clearing	Scant growth	Good green fluorescent growth

yellow of *Phytomonas phaseoli* and the browning of the media due to the action of *Phyt. phaseoli* var. *fuscans.* The yellow pigment of *Phyt. flaccumfaciens* may be so faint at times as to be scarcely visible, as on agars containing dextrose, and at other times or with other strains it may be as deep a yellow as in *Phyt. phaseoli.* The yellowish cream color of *Phyt. viridiflava* often is not observed. Older cultures show it better than do young cultures, and the sugar broths (dextrose and saccharose) bring out the color as a distinct orange, which may be confused with an indicator if such is present in the medium. The water-soluble green fluorescent pigment appearing on some media is a variable character, but *Phyt. vignae* var. *leguminophila* and *Phyt. viridiflava* have always produced this pigment on beef-extract agar (Bacto-peptone and Armour's beef extract). *Phyt. medicaginis* var. *phaseolicola,* while belonging to the green-fluorescent group, never produces the pigment on beef-extract agar. On the whole, however, it appears safer to base differences on physiological reactions in the media than on pictorial descriptions.

In a previous article (Burkholder, 1930), the writer pointed out that the genus Phytomonas is composed of at least three, and perhaps four, rather distinct groups of bacteria. It is interesting to note that each of the various bacterial pathogenes of the bean plant fall into one of the three distinct groups. *Phyt. phaseoli* and its variety *fuscans* belong to the *Campestre* group; *Phyt. flaccumfaciens* belongs to the *Stewarti* group, which contains the large proportion of Gram-positive and non-motile species; and the remaining organisms, *Phyt. medicaginis* var. *phaseolicola, Phyt. vignae* var. *leguminophila,* and *Phyt. viridiflava,* are green-fluorescent forms. While these green-fluorescent bacteria constitute a rather well-defined group of pathogenes, the individual members within are not clear-cut. Many of the pathogenes vary only slightly from the others, both in pathogenicity and in cultural reaction. In other words, there appear to be a great number of species, varieties, and forms, from which one might assume that the bacteria are not very stable. A condition exists here which is similar to that found in the genus Fusarium of the fungi. A discussion of the variability of the group is scarcely pertinent at this time, but it is interesting to note that the green-fluorescent bean pathogenes illustrate the point rather well. *Phyt. medicaginis* var. *phaseolicola* and *Phyt. vignae* var. *leguminophila* might have been given specific names, and no doubt would have been so treated by many investigators; but they exhibit such a close relationship to *Phyt. medicaginis* and *Phyt. vignae,* respectively, that it was considered best to show this in their name. In discussing the variability of the group, it should be pointed out also that the New York strain of *Phyt. vignae* var. *leguminophila* differs slightly from the New Jersey strain, and the English strain of *Phyt. viridiflava* is not identical with the Swiss strain, when pathogenicity is considered.

Differences in loss of virulence of the pathogene in pure culture

One point of difference among these bacteria which should be touched upon here, although it can scarcely come under the head of cultural reactions, is the variability in the loss of virulence, and even loss of pathogenicity, which exist among them. This fact has been mentioned in the individual descriptions, but it should be emphasized here where the peculiarities of each form are under discussion. The ability of the organisms to retain their virulence in pure culture varies with the species. *Phyt. flaccumfaciens* is an outstanding example of a pathogene that does not lose its virulence. Strains of this organism four and five years old are at hand which appear to have lost nothing of their ability to infect their host, or at least such a loss is not evident. *Phyt. medicaginis* var. *phaseolicola* is a second organism which may be placed in this class. One strain has been under observation for four years and still has the ability to kill young Red Kidney bean plants when these are inoculated. Certain symptoms, however, such as the stem lesions, are not so striking in appearance when produced by these old strains as when produced by a recently isolated strain, but the final result appears to be similar.

Strains of *Phyt. phaseoli* five and six years old will still cause definite pod spots. They do not, however, infect the vascular system and produce a wilt as rapidly as do recently isolated strains. Some old strains have apparently lost this ability, and a bean plant inoculated at a stem node with such an organism will show no generalized signs of the disease. *Phyt. viridiflava* and *Phyt. vignae* var. *leguminophila* lose their virulence very rapidly in pure culture. Continued inoculation of the bean plant with each, and reisolation of the organism, were necessary for inoculation experiments in order to have at hand a culture of the pathogene which showed any virulence. With *Phyt. viridiflava* the pathogenicity was at times even entirely lost.

In considering the loss of virulence in some of these species, one is forced to look upon this ability of organisms to produce disease as a rather delicate character, a variable one in many cases and one about which very little is known. The plant pathologist has been inclined, in naming species, to consider pathogenicity as a primary character. In this respect his position as plant pathologist has made him a little biased. The fermentation of a sugar is more stable, in many cases, than is the pathogenicity, and therefore is a more reliable character. We pay little attention to the sugar, however, and place importance on pathogenicity. It is there that our interest lies. It is possibly for this reason that many of these bacterial pathogenes have been overlooked in the past. All bacteria causing a blight in beans have been considered as one and the same. The problem, however, is more complicated than it appears on the surface, and probably the six forms discussed in this memoir are not all that attack beans. The cultural characteristics of the bacteria should be considered more carefully, and, when possible, differential media should be devised.

HOST RANGE

In the past many bacterial plant pathogenes have been described as occurring on certain hosts, as if this character definitely placed them. If sufficient cultural characteristics accompany such a report, no misunderstandings are likely to arise. If an insufficient description of the organism is given, however, synonyms will undoubtedly occur. Not only may a number of bacteria cause similar diseases in one species of plant, but the pathogenes themselves may have the ability to infect other species and genera of plants. This should be recognized, but just how far one should go in determining a host range for an organism it is difficult to state. The field is extremely large. When there is any doubt, however, concerning two or more species on different hosts, a certain amount of cross inoculation is necessary. With the bacterial pathogenes attacking beans, this is especially true. Here we have several green-fluorescent forms occurring on the common bean (*Phaseolus vulgaris*), and it is known that a number of green-fluorescent bacteria are pathogenic to other leguminous species. It is very desirable to know the host range of each organism. With the yellow species the confusion is not so great, but among these *Phyt. phaseoli* var. *sojense* is separated from *Phyt. phaseoli* mainly on its pathogenicity for a certain host.

A host range for a pathogene is important also for other than taxonomic reasons. For control measures it is very desirable to know what other plants may harbor the organism. Susceptible plants may then be eradicated, or their planting avoided.

Present knowledge concerning the susceptibility or the immunity of fourteen legumes to the six bean pathogenes under discussion here, is summarized in table 3. The results from many hundreds of inoculations

TABLE 3. HOST RANGE (LEGUME) OF SIX BACTERIAL PATHOGENES OF THE BEAN (PHASEOLUS VULGARIS)*

	Phytomonas phaseoli	*Phytomonas phaseoli* var. *fuscans*	*Phytomonas flaccumfaciens*	*Phytomonas medicaginis* var. *phaseolicola*	*Phytomonas vignae* var. *leguminophila*	*Phytomonas viridiflava*
Phaseolus vulgaris	+	+	+	+	+	+
P. lunatus	+	+	+	+	+	+
P. coccineus	+	+	+	+	−	+
P. angularis	−	0	+	−	0	0
Vigna sinensis	−	−	+	−	+	+
V. sesquipedalis	−	−	+	−	+	−
Glycine max	−	−	+	−	+ (pod)	+ (pod)
Lupinus polyphyllus	+	−	−	−	−	−
Dolichos lablab	+	−	+	−	+ (pod)	+
Pueraria hirsuta	−	−	−	−	+	+
Vicia faba	−	−	−	−	+	−
Pisum sativum var. *arvense*	−	−	−	−	−	−
Melilotus alba	−	−	−	−	−	−
Medicago sativa	−	0	0	−	0	0

* A plus sign denotes that infection has been obtained; a minus sign, that there is no infection; and a zero sign, that no inoculation experiments have been conducted. A plus sign followed by the word "pod" means that infection has been obtained on the pod only.

were considered in compiling this table. In all cases, care was taken to make certain that a virulent culture of the pathogene was being used. Nevertheless, when infections on a certain plant were not obtained, the experiment was repeated two and three times to make certain of the results. Even then, some unknown factor necessary for infection might have been absent. However, under the conditions maintained — a temperature of 80° C., and the use of young, rapidly growing plants as the host — it is felt that the data are very close to being correct.

It may be noticed that most of the organisms listed in table 3 show a distinct difference in their ability to infect many of the species of plants considered. *Phyt. phaseoli* var. *fuscans* and *Phyt. medicaginis* var. *phaseolicola,* however, have a similar host range as far as the table shows, and *Phyt. viridiflava* and *Phyt. vignae* var. *leguminophila* appear rather closely related in this respect.

DIFFERENTIATION OF THE BACTERIAL DISEASES FROM OTHER BEAN DISEASES NOT OF BACTERIAL ORIGIN

The bacterial diseases of the bean will not, as a rule, be confused with any other bean diseases of fungous or of virus origin. There are a few pitfalls, however, which should be considered here.

The only fungous disease that causes lesions on the stems, leaves, and pods of the bean plant is the anthracnose due to *Colletotrichum lindemuthianum.* The differences in the character of the symptoms of the anthracnose and of the bacterial blights have been emphasized repeatedly in numerous publications, and need not be described here. Any one who is at all familiar with plant diseases will be able to make the distinction between this fungous disease and bacterial blights.

The mosaic symptoms on the leaves of the bean plant produced by *Phyt. medicaginis* var. *phaseolicola* have been reported earlier in this memoir, and if these are observed alone some difficulty might arise in distinguishing them from the mosaic produced by the true virus disease. In examining infected plants as a whole, however, the separation of the two diseases on the basis of the other symptoms should be made very readily.

Fusarium martii f. sp. *phaseoli* will cause a stunting of the bean plant, a yellowing and dropping of the leaves, and sometimes a slight wilt. An examination of the roots of plants so appearing, however, would leave no doubt as to the organism causing the trouble.

Seedling wilts due to the bacteria are indistinguishable macroscopically from the hereditary seedling wilt described in a previous publication (Burkholder, 1926). If the wilt is due to bacteria, the microscope will reveal the cause. Furthermore, the hereditary wilt has never been observed except on hybrid plants.

Spots on bean pods due to sun scald are often confused with spots caused by bacteria. On certain varieties of beans, especially the garden varities,

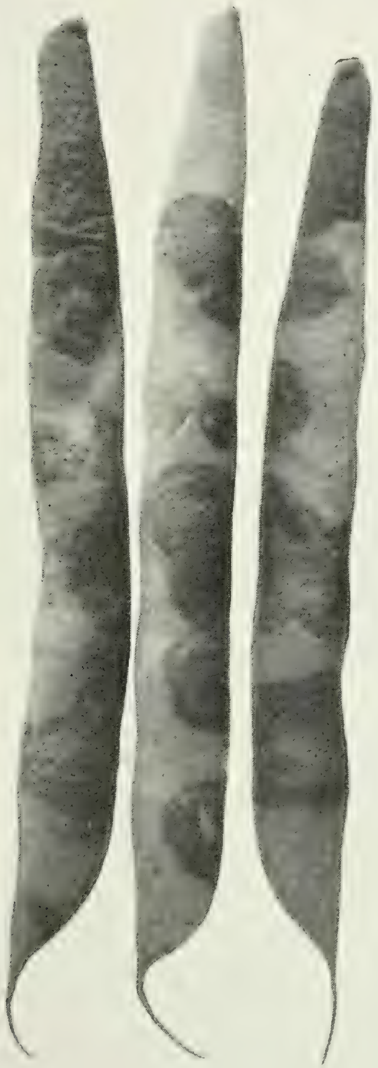


FIGURE 15. SUN SCALD ON BEAN PODS OF THE WHITE KIDNEY VARIETY

the similarity is so great that a microscope is essential in detecting the difference. However, there are certain dissimilarities between the two types of lesions which should be noted. Sun-scald spots always are found on the exposed surface of the bean pod, and they never show the water-soaked appearance that occurs with the more common bacterial diseases. In many cases, too, the sun scald appears on the pods over the seeds. These spots are somewhat sunken, and may be of any shape from rather irregular dashes to large spots more or less circular in outline which often anastomose. In color they are brown to red-brown, according to the variety of bean.

GEOGRAPHICAL DISTRIBUTION OF THE VARIOUS BACTERIAL DISEASES OF THE BEAN

The exact geographical distribution of the various bacterial diseases of the bean is difficult to determine, since it is only recently that more than one of these diseases has been recognized. In reports of plant-disease surveys, as a rule the cause of a blight of beans has been referred to *Phytomonas phaseoli.* Probably in the majority of cases this has been correct, but since there is seldom a description of symptoms of the disease accompanying such a report one can never be quite sure. Seldom if ever is an isolation of the causal organism made to determine its appearance in pure culture. In the following paragraphs the known geographical distribution of the six bacterial diseases of the bean here considered is given. The actual distribution is much more extended than that recorded here, since there have been reports of bacterial diseases of the bean in countries not mentioned in this account. From such reports, however, there would be some difficulty in determining which bacterial pathogene was involved.

More is known concerning the distribution of *Phyt. phaseoli,* the oldest recognized pathogene of the group, than for the other pathogenes. It is of widespread occurrence in the United States and Canada, and, being a seed-borne disease, probably is to be found in most bean-growing countries. Delacroix (1899) reported the disease from France shortly after Smith (1898) first described it as occurring in America. Little has been said, however, concerning the presence of this pathogene in France since Delacroix's report; and the writer was unable to find any bacterial disease in the bean fields in Normandy and those about Paris during the summers of 1924 and 1927. Welles (1922) reported the disease in the Philippines, and Picado (1924) reported a yellow bacterium, which probably was *Phyt. phaseoli,* causing a disease of beans in Costa Rica. The pathogene was isolated by the writer from leaves and pods collected in Bermuda in 1922, and from diseased pods collected in Switzerland in 1927.

Phytomonas flaccumfaciens, a less generally recognized pathogene, is found throughout the United States, as reported by Miss Hedges (1926). It has been isolated also, by the same investigator, from seed both from France and from Germany.

Phytomonas medicaginis var. *phaseolicola* was first observed in New York by the writer (Burkholder, 1926), and recently Miss Hedges (1928) has reported it from Montana, Wyoming, and Colorado in the West, and from Florida, Georgia, and South Carolina in the South. In Europe, M. K. T. Wieringe has isolated the pathogene in Holland, and the writer has obtained it from diseased bean pods collected in Switzerland.

Phytomonas phaseoli var. *fuscans* has been found in Switzerland causing a disease of the common bean, and Miss Hedges has isolated the organism from seed imported from South America.

Phytomonas vignae var. *leguminophila* has been found only in New Jersey and in New York.

Phytomonas viridiflava was isolated from pods collected in a market at Lucerne, Switzerland, and a similar organism was found causing a leaf spot of the scarlet runner bean near Canterbury, England.

CONTROL METHODS

The same control measures have been advocated for the various bacterial diseases of the bean, since the diseases are alike in many respects and since the life histories of the pathogenes are similar, all being seed-borne diseases. In New York the blights due to *Phytomonas phaseoli* and *Phyt. medicaginis* var. *phaseolicola* are the bacterial diseases which cause economic losses, and consequently are the ones with which control measures are especially concerned. The methods applied in holding in check these two diseases, however, appear to be effective against the other bacterial diseases of the bean also. These methods of control, which have been successful when applied, are two in number: first, the use when possible of bean varieties showing some resistance to the blights; and secondly, the use of disease-free seed in all cases. Rands and Brotherton (1925) have published a paper dealing with the varietal susceptibility of many varieties of beans to *Phyt. phaseoli* and *Phyt. flaccumfaciens*; and the writer (Burkholder, 1924) has reported on similar experiments with *Phyt. phaseoli*. Observations in the field and on inoculation work in the greenhouse leads to the belief that there is a close resistance correlation among the various bacterial diseases. Consequently, the use of any of the varieties listed in the above-mentioned articles as somewhat resistant will probably eliminate much of the trouble from all of these diseases. In New York, among the field beans or those frequently referred to as the dry shell beans, the Robust Pea bean shows considerable resistance to these bacterial diseases. Also, in the fields the blights seldom cause much damage to Yellow Eye, and White Marrow is not extremely susceptible. With reasonable precautions in selecting the seed to be planted, one should have little trouble with these varieties.

The roguing of parts of fields, and the use of seed from such parts for planting in the following year, has been practiced with success with Perry Marrow. It should be remembered, however, that this procedure can be carried out with greater ease in some seasons than in others. In a season favorable to the blight, when the diseases spread rapidly, some difficulty might be encountered. Ordinarily, however, the method should be beneficial. It should be emphasized that it is only among the varieties that show some resistance that roguing should be applied. This method of control with Red Kidney or an equally susceptible variety probably would be of little avail. The diseases, especially the halo blight, spread so rapidly through a field of Red Kidney beans that it would be impossible to remove all diseased plants and have any plants left.

The use of seed from disease-free fields is being practiced in New York by many of the better growers. A field in which there are no diseased seedlings to act as a source of infection remains reasonably free from the bacterial blights, since infection from outside comes in late and has no opportunity to do much damage. When it is impossible to find disease-free fields, the use of seed from fields which show only a small percentage of infected plants probably is more desirable than the use of seed from fields where the percentage of diseased individuals is high. Nevertheless, a very small percentage of blight in one year in a field of Red Kidney beans may develop to 100 per cent in the second year. This may be the case also with other extremely susceptible varieties.

It has been found, recently, that Red Kidney beans grown in California are entirely free from the bacterial blights, and that such seed brought to New York has produced healthy fields the first year. Ultimately, under our conditions the seed becomes contaminated. The length of time for this to happen would depend, no doubt, on the care taken of the seed. If planted next to or near a field of New-York-grown seed, it probably would become infected very quickly. If isolated in a field from all other beans, it would be safe for use as seed so long as no disease appeared in the field.

The demand for California-grown Red Kidney seed in New York has become very great, and there is on the market considerable seed labeled as being from California which probably never came from there. At least much of the seed is infected with bacterial pathogenes. The bean-growing areas of California are not known to the writer, and it is possible that certain sections do not produce blight-free seed. The location and study of areas able to produce disease-free seed would be of great benefit to the bean industry.

Spraying or dusting field beans for the bacterial blights, or even for the fungous diseases, is not to be recommended under New York field conditions. There are several reasons for this. The selection and care of disease-free seed and the use of disease-resistant varieties are simple methods of control, and, when employed with good cultural practices, are satisfactory. Spraying and dusting are cumbersome, disagreeable, and expensive processes even when they are effective, and the bacterial blights of the bean are not of sufficient enphytotic character to warrant their expense. That the expense would be considerable is certain. Numerous applications would be necessary, since the bean plant is susceptible to the blights at all periods in its life and also is a rapidly and almost continuously growing plant. This would necessitate applications from the seedling stage until the pods were mature. Furthermore, the bean-blight problem in New York is not complicated with an insect problem which frequently makes spraying or dusting necessary. One has only the disease control to consider. Again, one must take into consideration how much damage a spray machine would do to the bean plants. Such varieties as Marrow, Medium, Pea, and Yellow Eye are of indeterminate growth habits, and not only the vines, but the pods on them would be destroyed. Even cultivators cause damage to beans, especially when the plants reach the blossoming and podding stages, and it is one of the better practices in bean culture to prepare the land so thoroughly that few cultivations are necessary.

How long the pathogenes will live in old bean vines in the fields is not known. The ability of *Phyt. phaseoli* and *Phyt. flaccumfaciens* to live for many years in a dry condition in leaves and seed, and of *Phyt. phaseoli* to live for at least a year in the soil, shows that these two organisms are rather resistant to their environment. A clean-up of old vines is desirable as a general sanitary measure, and probably would reduce chances of infection where rotation of the bean crop is short.

SUMMARY

This memoir reports an investigation of the bacterial diseases of the common bean (*Phaseolus vulgaris*). Six distinct diseases are recognized, the causal organisms of which are as follows: *Phytomonas phaseoli*, *Phyt. flaccumfaciens*, and *Phyt. medicaginis* var. *phaseolicola*, which have hitherto been reported upon; and three newly described organisms, *Phyt. phaseoli* var. *fuscans*, *Phyt. vignae* var. *leguminophila*, and *Phyt. viridiflava*.

Careful and extensive inoculation experiments have proved the pathogenicity of each organism.

The symptoms of each disease on the bean plant are described in full and their similarities and differences are noted.

Various phases of the life history of the organisms are described. *Phyt. flaccumfaciens* is primarily a vascular parasite; *Phyt. phaseoli*, *Phyt. phaseoli* var. *fuscans*, and *Phyt. medicaginis* var. *phaseolicola* may or may not be vascular parasites; *Phyt. vignae* var. *leguminophila* under certain conditions may be found in the xylem; but *Phyt. viridiflava* cannot be considered a vascular parasite.

The morphology and the cultural characteristics of each pathogene are given. *Phyt. viridiflava*, *Phyt. vignae* var. *leguminophila*, and *Phyt. medicaginis* var. *phaseolicola* belong to the green-fluorescent group of bacterial plant pathogenes; *Phyt. phaseoli* and its variety *fuscans* belong to the *Campestre* group; and *Phyt. flaccumfaciens* belongs to the *Stewarti* group.

The pathogenes vary in their host range over a dozen leguminous plants which were used in a series of inoculation experiments.

Control measures for the diseases are discussed.

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The following list of articles by no means contains all the references to the bacterial diseases of the bean. It does contain the more important ones, together with certain foreign references which would help in determining the distribution of the various diseases. American literature is full of short descriptions and paragraphs mentioning bacterial affections of this crop. Such articles are disregarded here unless they have contributed something to our knowledge of the subject. Other articles and books listed, which do not have a direct bearing on the diseases under discussion, contain descriptions of methods and technic which have been used in this work.

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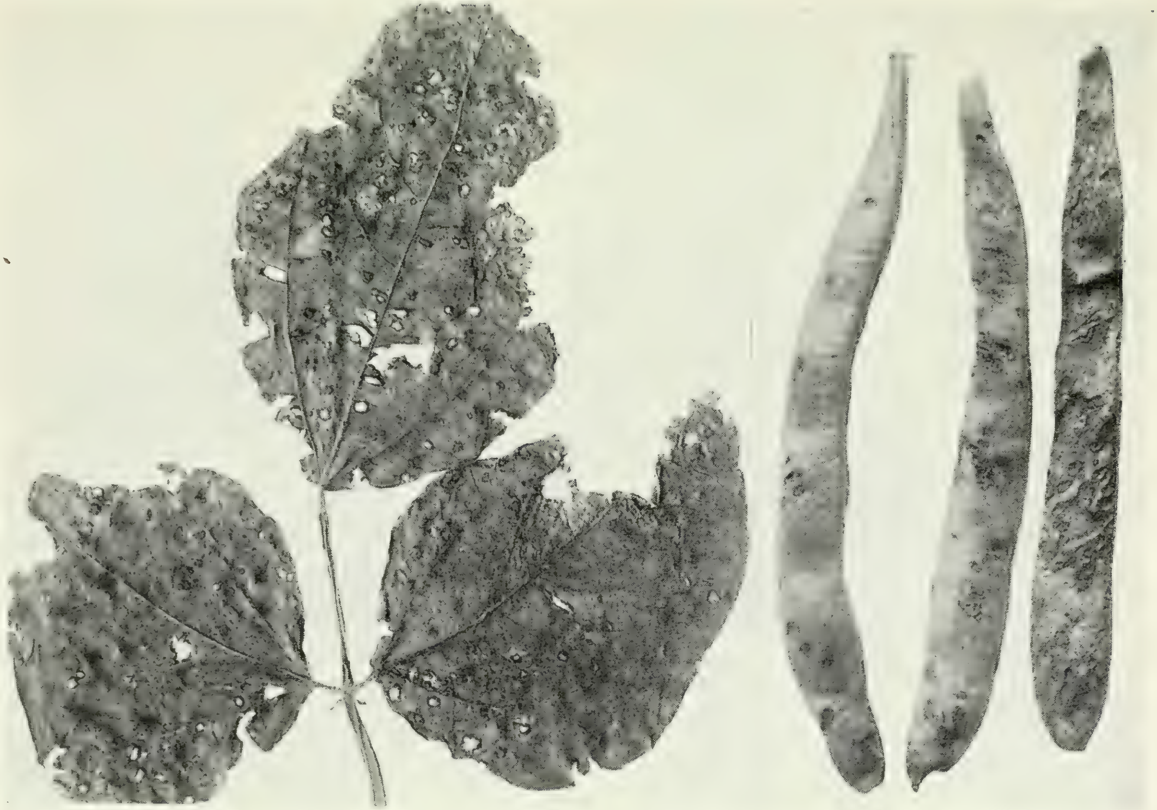
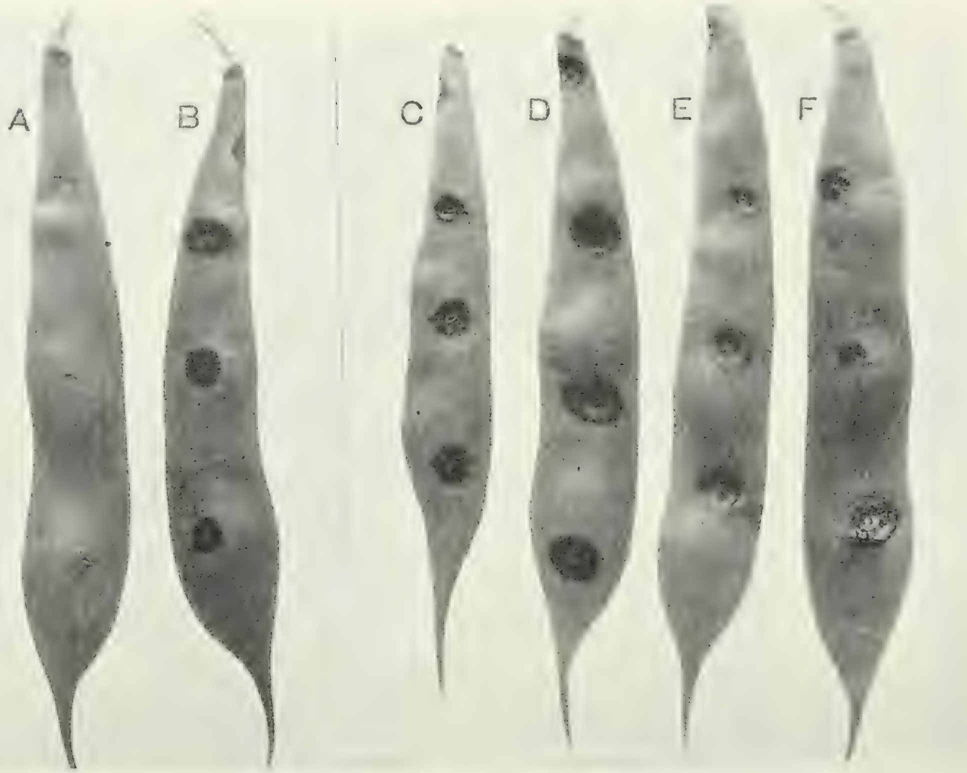
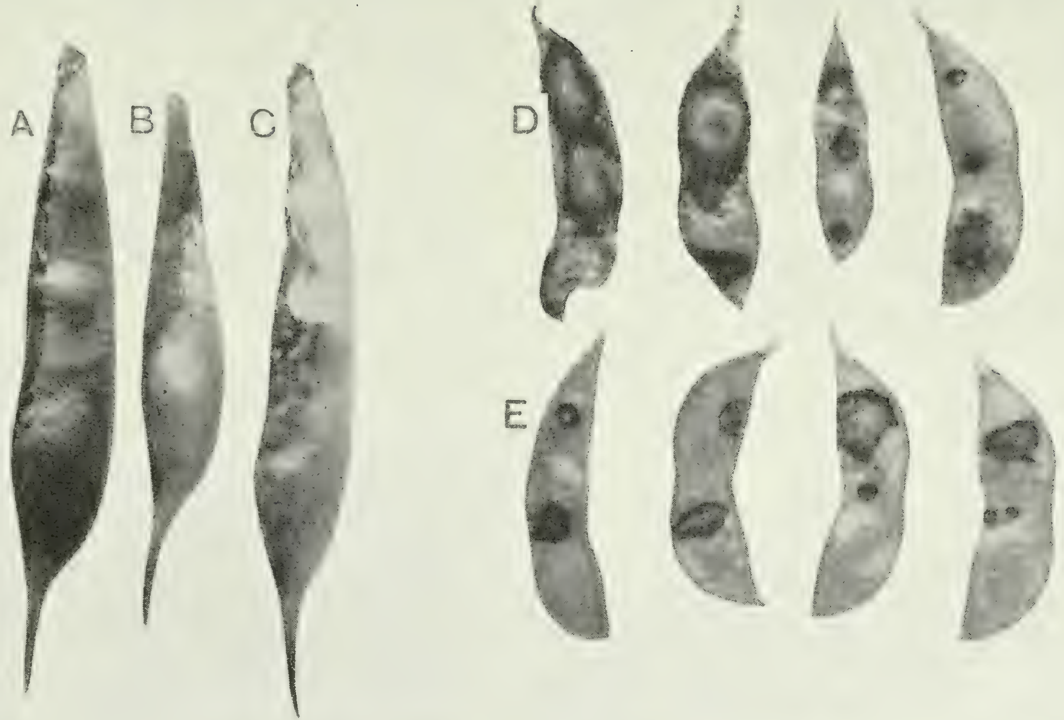


LEAF OF A GARDEN VARIETY OF BEANS, WITH SMALL, SLIGHTLY RAISED LESIONS CAUSED BY PHYTOMONAS PHASEOLI, AND PODS FROM THE SAME PLANT



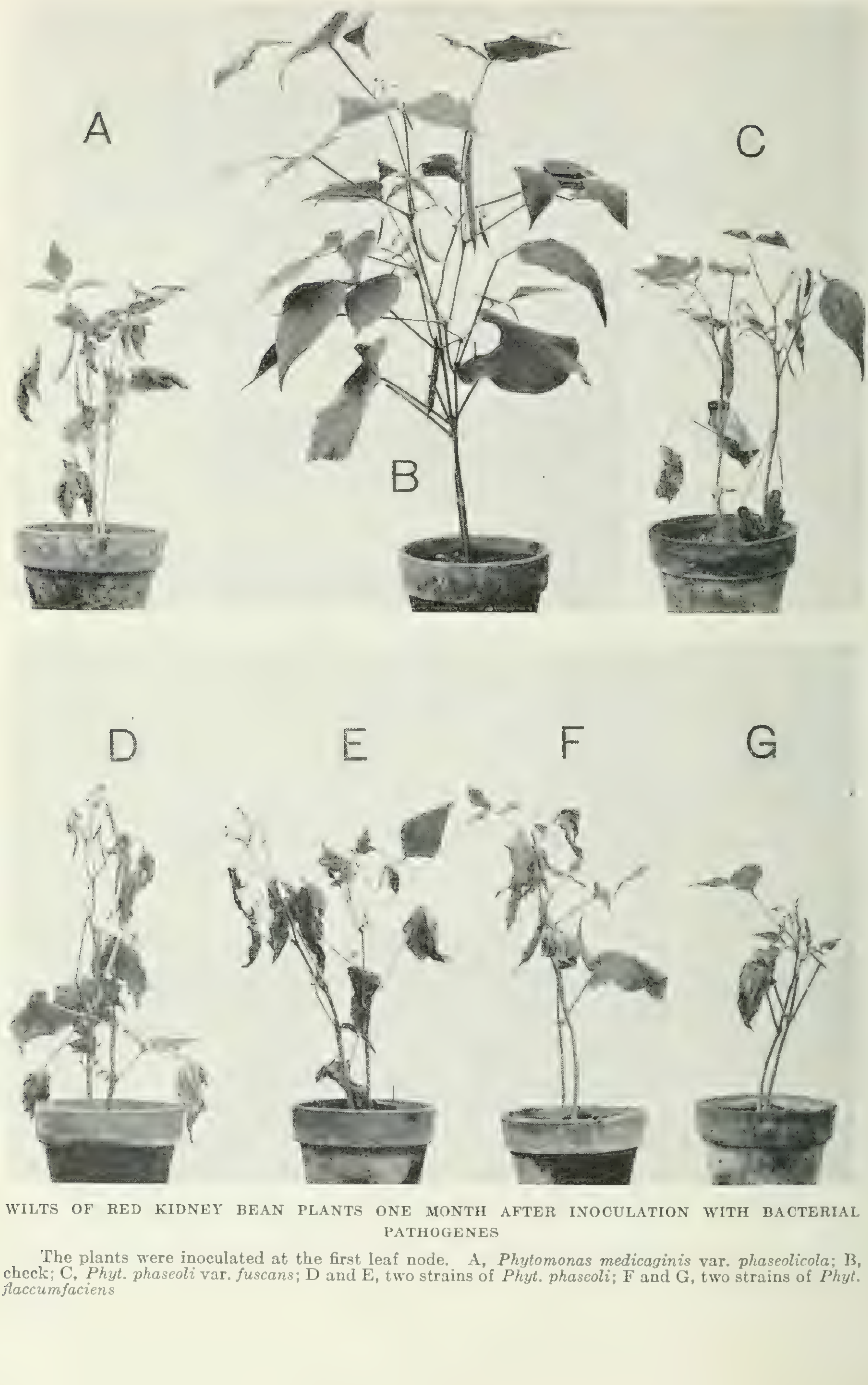
RED KIDNEY BEAN PODS SEVEN DAYS AFTER INOCULATION WITH BACTERIAL PATHOGENES

The pods were injured at the time of inoculation. A, *Phytomonas flaccumfaciens*; B, *Phyt. medicaginis* var. *phaseolicola*; C, *Phyt. vignae* var. *leguminophila* (New York strain); D, *Phyt. viridiflava* (Swiss strain); E, *Phyt. vignae* var. *leguminophila* (New Jersey strain); F, *Phyt. viridiflava* (English strain)



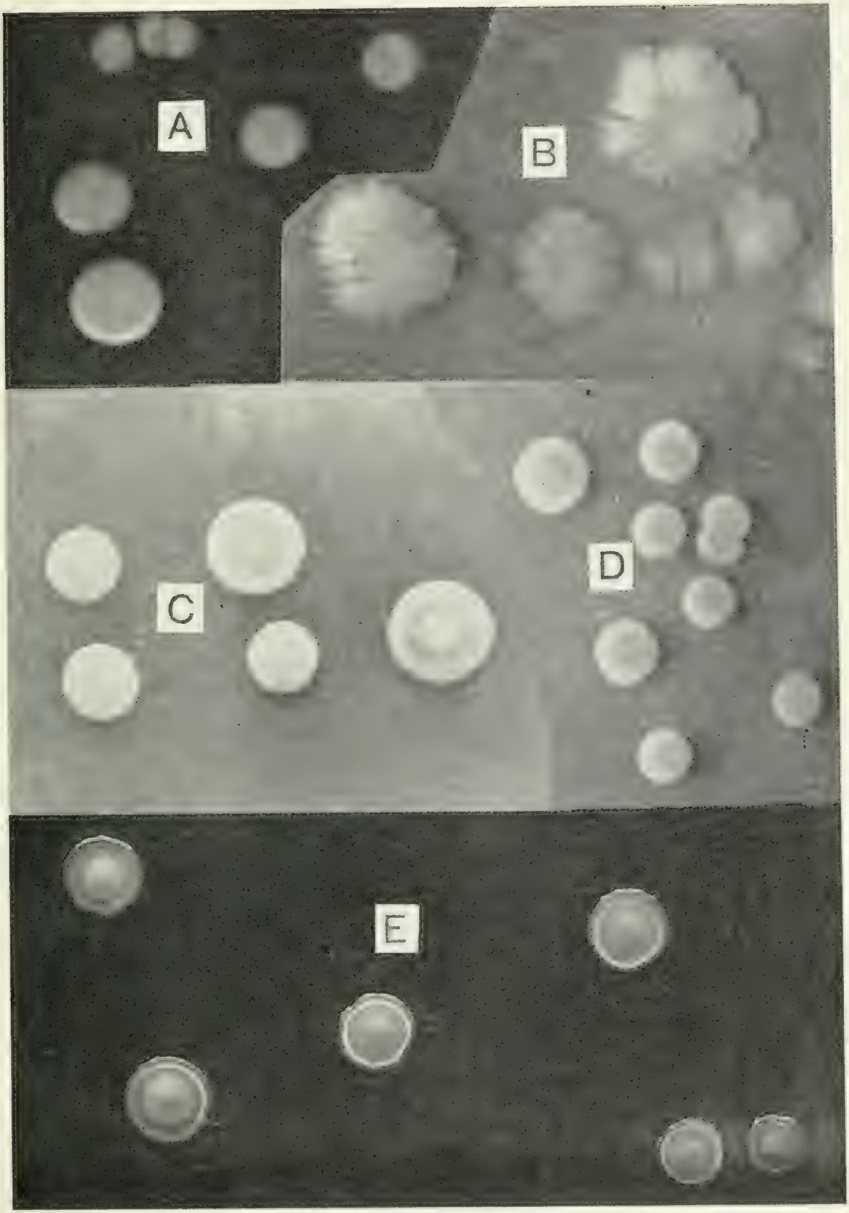
A TO C, SUTURE LESIONS ON RED KIDNEY BEAN PODS DUE TO INOCULATING THE PEDICEL WITH BACTERIAL PATHOGENES
D AND E, SOYBEAN PODS SHOWING LESIONS DUE TO ARTIFICIAL INOCULATIONS WITH BACTERIAL PATHOGENES

A, *Phytomonas medicaginis* var. *phaseolicola*; B, *Phyt. phaseoli*; C, *Phyt. phaseoli* var. *fuscans* (this type of lesion is often caused also by *Phyt. flaccumfaciens*); D, *Phyt. vignae* var. *leguminophila*; E, *Phyt. viridiflava*



WILTS OF RED KIDNEY BEAN PLANTS ONE MONTH AFTER INOCULATION WITH BACTERIAL PATHOGENES

The plants were inoculated at the first leaf node. A, *Phytomonas medicaginis* var. *phaseolicola*; B, check; C, *Phyt. phaseoli* var. *fuscans*; D and E, two strains of *Phyt. phaseoli*; F and G, two strains of *Phyt. flaccumfaciens*



SURFACE COLONIES OF VARIOUS BACTERIAL PATHOGENES OF THE BEAN

A, *Phytomonas vignae* var. *leguminophila*; B, *Phyt. viridiflava*; C, *Phyt. phaseoli* var. *fuscans*; D, *Phyt. flaccumfaciens*; E, *Phyt. medicaginis* var. *phaseolicola*

A to D, Colonies seven days old, temperature 27° C., beef-extract agar (pH 7.0), × 2. E, Same conditions except that colonies are fourteen days old and agar is slightly acid

MEMOIR 128 MAY, 1930

CORNELL UNIVERSITY
AGRICULTURAL EXPERIMENT STATION

STUDIES ON FUNGICIDES

I. CONCEPTS AND TERMINOLOGY

H. H. WHETZEL AND S. E. A. McCALLAN

II. TESTING PROTECTIVE FUNGICIDES IN THE LABORATORY

S. E. A. McCALLAN

III. THE SOLVENT ACTION OF SPORE EXCRETIONS AND OTHER AGENCIES ON PROTECTIVE COPPER FUNGICIDES

S. E. A. McCALLAN

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STUDIES ON FUNGICIDES

I. CONCEPTS AND TERMINOLOGY

H. H. WHETZEL AND S. E. A. MCCALLAN

There is now under way or projected, in the Department of Plant Pathology at Cornell University, a series of investigations directed toward the solution of fundamental problems in fungicidal efficiency. It is proposed to publish the results of these investigations as units in a continuing series under the general title *Studies on Fungicides.* As introductory to the series, this first paper sets forth some concepts and terminology which appear basic and indispensable to a rational discussion of hypotheses and results to be presented in succeeding papers.

The term *fungicide* is commonly employed to designate any chemical element or compound which is toxic to fungi. It is a more restricted term than *germicide,* and is coordinate with *bactericide, insecticide, nematocide,* and the like. While commonly restricted in its application to chemical elements or compounds, it may perhaps be extended legitimately to include such physical agents as light, heat, or other forms of radiant energy. In this paper it is employed in the more commonly restricted sense. Only chemical fungicides are dealt with.

Since the toxic constituents of fungicides can act on the fungi only by diffusing into the protoplasm, it is evident that they must eventually become soluble. Many of our commonly used fungicides are immediately soluble in water. It is generally recognized, however, that certain of our standard fungicides, notably bordeaux mixture and sulfur dust, are not sufficiently soluble in pure water to exercise their fungicidal properties. A number of hypotheses have been advanced to account for the evident solution of the toxic constituents of these fungicides after they have been applied to plants. Certain constituents of the atmosphere, excretions from the plant itself, and excretions from the germinating spores, each have been held by investigators to be the dissolving agent.

Therefore, on the basis of their state in water, fungicides may be divided into two classes: (1) those soluble in water, that is, those in which the toxic principle acts directly and immediately upon the living protoplasm of the fungus; and (2) those essentially insoluble in water, that is, those in which the toxic principle can act effectively only on being liberated by some other agent than water. While all chemical elements or compounds are probably soluble in water to at least a very slight degree, the term *insoluble* is here used in the sense that the toxic principle is of itself not sufficiently soluble in water to inhibit or terminate infection by the pathogene.

Based on its function in any given case, a fungicide acts as a *protectant*, a *disinfestant*, or a *disinfectant*. It functions as a protectant, when applied to the suscept before inoculation, that is, before the inoculum has arrived in the infection court (for example, in spraying apples for scab); as a disinfestant, when applied after inoculation but before infection, that is, after arrival of the inoculum but before the pathogene has established itself in the suscept (for example, in treating seed wheat for stinking smut); as a disinfectant, only when applied after infection has taken place (for example, in spraying plants already affected with powdery mildews).

Fungicides that act to control the disease when they are applied after the pathogene has become associated with the suscept (that is, after inoculation or infection), are now commonly designated as *disinfectants*. We can speak accurately of disinfection, however, only when the fungicide acts on the pathogene after it has established pathogenic relations with the suscept. When the toxic action of the fungicide is exerted on the pathogene while it is merely in physical association with the suscept, as in the case of many seed-infesting pathogenes, it is more nearly precise to designate its action as *disinfestation*.

One has to consider, moreover, in this connection the whole problem of terminology in the matter of fungous infestation of the soil or other nonliving harborers of fungous pathogenes. The almost universally used expressions, " diseased soil," " sick soil," " infected soil," " inoculated soil," " soil inoculation," and the like, are inaccurate and unscientific in the light of our present ideas and concepts of disease phenomena. Disease is an injurious physiological process, a response on the part of the suscept to pathogenic irritation. Even if one includes (as one usually does) in the concept of disease, the chemical reactions of the dead tissues of timber to the activities of decay-inducing fungi, one certainly has no similar relationship in mind between phytopathogenic fungi and the soils in which they are present. Strictly speaking, infection is pathogenic irritation. Only living things can be infected. Soil, therefore, cannot be *infected*, dictionaries to the contrary notwithstanding; it may only be *infested* or *contaminated*. Obviously, since soil cannot be infected or diseased in any reasonable sense of these words, it cannot be " inoculated." Only living things that presumably possess the power to respond to pathogenic agents, can be inoculated. One can contaminate or infest a soil with pathogenes; one cannot inoculate it. The extensive use of " inoculate " to designate the planting of organisms on culture media is equally faulty; " planting " or " seeding " is better usage.

It follows, therefore, that one may not logically speak of " disinfecting " the soil, or of a " soil disinfectant." The terms *disinfestant* and *disinfestation* express more nearly accurately and specifically the concepts involved. The writers advocate the use of the terms *disinfect* and *disinfectant* only in those cases in which the destruction of the pathogene results in the cessation of infection (for example, in the treatment of oat seed with formalin for control of loose smut). Here the seed is actually infected (Gage, 1927). Where destruction or elimination of the pathogene from mere physical association with the suscept or other harborers is involved, the terms *disinfest*, *disinfestant*, and *disinfestation* are to be preferred (for example, treating wheat seed for control of stinking smut, or drenching soil with a solution of formaldehyde to eliminate phytopathogenic organisms).

Water-soluble fungicides, with few exceptions, function as either *disinfestants* or *disinfectants*. They are usually effective only in cases of infestation, though sometimes they may actually disinfect, that is, destroy the pathogene after it has established pathogenic relations with the suscept. The use of lime-sulfur or potassium sulfide to destroy the powdery-mildew fungus already established on roses or other plants, is a good example of disinfection by water-soluble fungicides. There appear to be few water-soluble fungicides employed in plant-disease control which are capable of penetrating the tissues of the plant to destroy an invading pathogene without economic injury to the suscept. The action of formaldehyde in the case of loose smut of oats (Gage, 1927), and that of borax or boric acid in the disinfection of oranges infected by Penicillium (Fawcett and Lee, 1926:361), appear to be cases in point. The water-soluble fungicides most commonly employed for purposes of disinfestation in plant-disease control are formaldehyde, some mercury compounds, and certain copper compounds.

A good disinfestant is (a) toxic to the infesting pathogene, (b) practically harmless to the infested organs of the suscept at effective concentrations, (c) innocuous to the soil or other non-living harborers infested with the pathogene, (d) easy of application, and (e) economical.

Where used as a disinfectant, the fungicide must be (a) able to penetrate to the pathogene if that is within the tissues of the suscept, (b) toxic to the infecting pathogene at a concentration practically harmless to the tissues of the suscept, (c) easy of application, and (d) economically profitable in control of the disease.

Water-insoluble fungicides commonly function as *protectants*. They are usually effective only when applied to the plant before it has become inoculated with the spores of the pathogene. Bordeaux mixture is the classical protectant. Its protective function was clearly perceived by its discoverer, Millardet (Crandall, 1909:205). Next to bordeaux mixture, sulfur in its elemental state, either in suspension (dry mix) or as dust, is perhaps the most widely used protectant. Lime-sulfur, a water-soluble fungicide, at first thought might be assumed to function directly as a protectant. A moment's reflection will at once discover the fallacy in this assumption. It is chiefly after the lime-sulfur has dried and has been converted by the action of the carbon dioxide of the air into other compounds and free sulfur, that its protective function is exercised (Haywood, 1907). The elemental sulfur precipitated from the lime-sulfur chiefly functions as the protectant.

It has long been known, though not generally appreciated, that certain essentially water-insoluble arsenical compounds — for example, arsenate of lead and arsenate of lime — exhibit marked protective action against the apple-scab pathogene, *Venturia inaequalis* (Butler and Doran, 1928). The writers and T. C. Loh (Whetzel, McCallan, and Loh, 1929) have recently reported that calcium arsenate exhibits marked specificity against *Alternaria panax*, a pathogene but slightly susceptible to the toxic action of copper or sulfur protectants.

That insoluble fungicides may sometimes act as disinfestants, or even as disinfectants, is not to be denied. The elimination of powdery mildews by applications of sulfur dust to infected plants may be looked upon as a case of disinfection by an insoluble fungicide. Such cases are, however, few and exceptional.

A good protectant is (a) practically insoluble in pure water, (b) highly adhesive to the surface of the suscept organs to be protected, (c) active only in the presence of some solvent, (d) essentially harmless to the suscept, (e) miscible with insecticides if desired, the efficiency of neither being impaired nor the suscept endangered, (f) easy of application, and (g) economical.

It is evident, then, that water-soluble fungicides act chiefly as disinfestants or disinfectants, while water-insoluble fungicides function, with few exceptions, as protectants.

In the foregoing presentation, the writers offer what they hope may appeal to plant pathologists as a much-needed clarification of the chaotic state of the terminology of our current concepts of fungicides and fungicidal phenomena. It is believed that we have now reached a point in the development of the science of plant pathology where a revision in our terminology, consistent with the present state of our concepts in this field, will conduce to more nearly accurate and more logical thinking. It may be argued that general usage justifies the application of terms in a sense contrary to their strict meaning. This position can hardly be maintained, however, in the face of the current confusion in our terminology of this phase of plant pathology. Neither is such a position tenable on the ground of want of words adequate to convey the specific concepts involved. Even where such words are not already available, the language offers abundant material for acceptable coinage. The concepts and terms presented in this paper have long been employed by the senior author in his college course on the principles of plant-disease control.

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STUDIES ON FUNGICIDES

II. TESTING PROTECTIVE FUNGICIDES IN THE LABORATORY

S. E. A. McCallan

The laboratory testing of fungicides to determine their toxic properties has gained ground rapidly during recent years. The growing interest in this matter is doubtless due, in part at least, to the ever-increasing number of proprietary compounds on the market, a condition which makes field testing all but prohibitive in time and labor. In view of these facts, a short discussion at this time relative to the general scope of fungicidal tests in vitro, and the presentation of a method developed in the Laboratory of Plant Pathology at Cornell University and long in use there, may be serviceable to other workers. Such a discussion is presented in the following pages, and will serve as an introduction to the technic employed in certain studies on fungicidal action which constitute the third paper of this series.

Experience here has demonstrated, over a period of years, that the toxicity of a protective fungicide can be most readily and effectively determined in the laboratory. When the difficulties involved in measuring the other requirements of a good protectant (page 6) under the unnatural conditions of the laboratory or even of the greenhouse are considered, it is probable that testing under natural field conditions will give, for the present at least, the most reliable results for such factors as adhesiveness, toxicity to the plant, and the like.

A study of the literature on the nature of fungicidal action shows that, in general, one of three hypotheses usually has been advanced to explain the liberation of the active fungicidal principle from the insoluble protectant. These hypotheses are: (1) that this is accomplished by means of atmospheric agencies, especially rain, dew, and the carbon dioxide in the air; (2) that it is accomplished by the suscept, especially by the excretions from the organs to be protected; and (3) that it is accomplished through a solvent action exerted by the fungus spores themselves upon the protectant.

The literature relative to the first hypothesis is much in conflict, and experiments by the author, which are still in progress, show that, at least for certain common protectants, the rôle played by atmospheric agencies is almost negligible. Convincing evidence to substantiate the hypothesis of suscept excretions is lacking in the literature. The data on the hypothesis of spore excretions are very limited and are somewhat in

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Author's acknowledgments. The writer desires to acknowledge his indebtedness to all those workers of the Laboratory of Plant Pathology at Cornell University, both past and present, who have been instrumental in the development of the method of testing fungicides here described. Especial thanks are due to Professor H. H. Whetzel, of the Department of Plant Pathology, for the valuable advice rendered by him in the progress of these studies.

conflict. However, the author's experiments strongly support the third hypothesis. The evidence for this is presented in the next paper of this series.

It is evident that if fungicidal action involves essentially only a relationship of spore and protectant, the laboratory testing of fungicides, confined also to this relationship, may be expected to be both reliable and practicable. The advantages of laboratory tests lie in the saving of time, expense, and labor, and in the more definite control of environmental conditions. A set of tests may be made in a month or two which under field conditions would require several years. Thus, from many possibilities a few probabilities may be selected for actual field testing.

It must be emphasized that in the testing of fungicides the determination of their toxicity is of primary importance. The toxicity having been determined in the laboratory, the investigator is then in a position to proceed to the field for tests of adherence and injury to the plant. Spraying or dusting experiments on a large scale in the field must serve as the final test in the evaluation of any fungicide.

In the preparation and selection of fungicides, laboratory testing is playing an increasingly important rôle. Not only in many phytopathological research laboratories does this constitute a significant part of the program, but also in commercial laboratories.

The practicability, advantages, limitations, and uses of laboratory tests of fungicides have been outlined. What, then, is necessary in a reliable method of testing the toxicity of protective fungicides in vitro? First of all, it must duplicate, in so far as possible, actual field conditions. Some methods depart from this requirement to a large degree. Wallace, Blodgett, and Hesler (1911:167) state:

> It is true that many experiments have been conducted to determine the effect of certain fungicidal solutions on spore germination. In most such cases, however, the spores were placed directly in a solution or mixture of the fungicide. The results, therefore, must be considered as of scientific interest rather than as serving to indicate what these same substances will do after being sprayed and dried on the plant. The latter is the condition in which fungicides, with very few possible exceptions, must prevent infection.

Other considerations call for a method that is accurate and quick, with a simple technic, and capable of wide application.

DEVELOPMENT OF THE METHOD

In 1910 Reddick and Wallace published a brief note in *Science*, entitled *On a Laboratory Method of Determining the Fungicidal Value of a Spray Mixture or Solution.* This was the first published record of this method, the fundamental principle of which was developed by Dr. Reddick in connection with some class work during the winter of 1908 (Wallace, Blodgett, and Hesler, 1911). A few months later, Wallace, Blodgett, and Hesler (1911) devoted considerable space to a discussion of the method.

In explanation they say, in part (page 167 of reference cited): "We must, therefore, expect that progress in the discovery of new fungicides or beneficial modifications of old ones will be slow until some method is adopted whereby the *fungicidal properties* of various substances can be *studied in the laboratory*." Since that time the method has been more or less continuously employed in this laboratory by various workers who have introduced modifications and inprovements at different periods. During the past three years the author has had occasion to use the method very extensively. Naturally, some new problems have presented themselves, and, as obstacles have arisen, attempts more or less successful have been made to overcome them. As now developed, it is believed that this method of testing approaches actual field conditions more nearly than does any other described in the literature.

TECHNIC

The technic of the method as now in use is as follows:

The fungicide to be tested is sprayed or dusted on standard 3x1-inch clean glass slides which are conveniently placed on a tray. Every effort must be made to get an even and well-distributed layer of the fungicide. (The matter of application is presented in more detail later.) With such dusts as copper-lime, which should be dusted only on wet foliage, the slides must first be sprayed with distilled water until they are coated with fine, uniform droplets. The dust is then immediately applied. Liquid sprays are left exposed to the air and allowed to become completely dry. This will usually take several hours. The slides are then ready to be placed in the moist chambers.

Moist chambers are used in order to keep, without evaporation, the drops of water in which the spores are placed to germinate. These chambers have been found more suitable than petri dishes, since four slides may be placed under exactly the same conditions. Also, the air relations are better. In order that the chambers may be sealed with water, they are inverted for use. A satisfactory size of moist chamber is 7 by 20 centimeters. A piece of clean filter paper is placed in the bottom of the chamber. This tends to hold the moisture and to prevent the slide-holding racks from displacement, and provides an excellent background for purposes of observation. The slides must be held above the moist filter paper or the water. To accomplish this, glass slide racks are used, one for each chamber. These may be easily constructed of glass tubing bent to form a U. The length of the rack should be sufficient to easily hold the four slides parallel to one another, and yet allow room for the chamber cover to be placed in position (figure 1).

A suspension of the spores to be tested is prepared by carefully washing them from the substrate into the particular kind of water desired. If

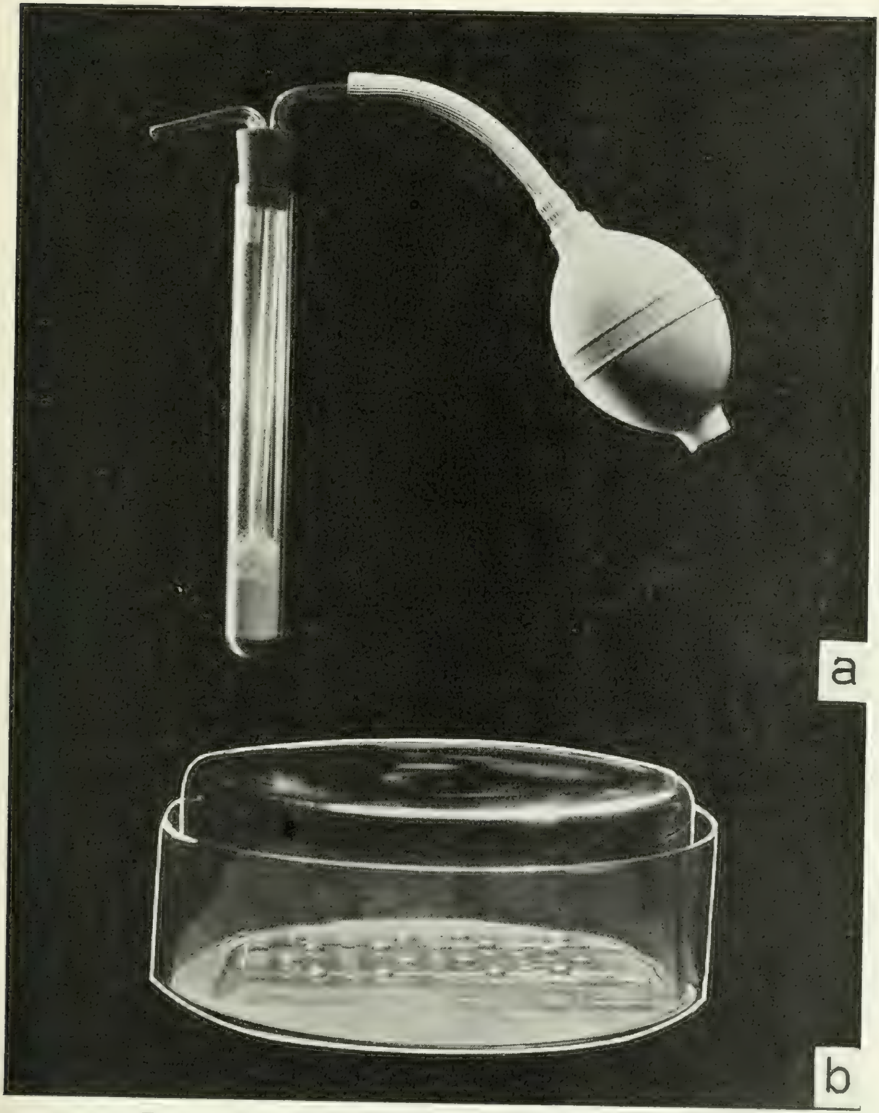

FIGURE 1. TEST-TUBE DUSTER (a) AND MOIST CHAMBER (b)

The moist chamber is equipped with filter paper, slide rack, and slides, for a spore-germination test. The spores are suspended in the drops of water on the slides. The chamber has not yet been sealed with water necessary, the spore suspension is filtered through cheesecloth or cotton to remove bunched spores and pieces of mycelium or of substrate. The spore concentration of the suspension is regulated by dilution until, as ascertained by examination of representative drops under the microscope, the desired concentration has been attained. Test tubes have been found most suitable for holding the suspension, since with these it may be readily agitated and kept uniform. By means of a pipette that will deliver about twenty drops to a cubic centimeter, the spore suspension is pipetted as drops on the slides. The optimum number of drops per slide is four. A greater number tend to run together, and a less number results in a decrease in manual efficiency and loss of replications. There will thus be sixteen drops to a chamber. To insure that the drops contain as representative a number of spores as possible, it is well to draw a fresh pipette-full of spore suspension for at least every two slides. Only one fungicide should be placed in a chamber; experience has taught the necessity of this. The moist-chamber cover is put in place, and water is poured around it into the bottom. This seals the chamber and preserves an atmosphere approaching saturation. Drops may be kept in this manner for a number of days before complete evaporation results. A moist chamber completely equipped and with the drops of spore suspension present, but just prior to the final act of sealing with water, is illustrated in figure 1, b. Controls are of course maintained, drops of spore suspension being placed on clean blank slides. One moist chamber containing four slides will usually constitute a satisfactory control. Thus the spores are allowed to germinate undisturbed at their optimum temperature. When the time has elapsed (determined for the particular fungus) in which a maximum number of spores in the control have germinated, producing vigorous germ tubes, the results may be taken by recording the percentage of germination and the general vigor of the germ tubes.

IMPORTANT FACTORS

In conducting these tests there are a number of factors that must be taken into consideration. For convenience these may be discussed in the order in which they appear, and may be classified under the following general headings: clean glassware, standardization of the fungus and of the conditions for spore germination, and application of the fungicide. Disregard of any of these factors gives unsatisfactory results.

CLEAN GLASSWARE

It is very essential that all the glassware be clean. The free use of chromic-acid cleaning mixture is necessary, for the slides must be chemically clean. The cleaned slides should be stored and protected from dust. At the end of each experiment, the moist chambers and the slide racks must be thoroughly washed with water. The use of dirty or used moist chambers markedly affects the results of experiments.

The necessity for sterile conditions has not been felt in the use of this method. Interest is only in germinating the spores, and if ordinary precautions are taken to prevent undue contamination it has been the writer's experience that satisfactory results can be obtained. It is, of course, entirely possible that with spores of certain fungi sterile conditions will be necessary.

Other than the fungicide, no foreign substance should be placed in the chamber. Clark (1899), Duggar (1901), and Melhus and Durrell (1919) have noted that the presence of such foreign substances as carbon, paraffin, and vaseline has stimulated spore germination. Glue and balsam used to cement certain types of slide racks have greatly influenced spore germination in this laboratory. The glass-tube type of rack now used carries no foreign material and can readily be washed.

STANDARDIZATION OF THE FUNGUS AND OF THE CONDITIONS FOR SPORE GERMINATION

These factors are of paramount importance, though unfortunately often disregarded. It is very essential, if one plans to work with any fungus on a large scale, that it should be " standardized "; that is, the optimum factors for spore germination should be determined, and all conditions controlled, in so far as possible. Unless the control spores germinate consistently, no significant comparisons of toxicity can be made. This is because spores of lowered vitality are greatly influenced by any adverse condition, such, for example, as treatment with a toxic agent. The result of this is a low germination out of all proportion to that of the control when compared with treated and control spores of greater viability.

Source of spores

Ideally, perhaps, the spores should be obtained from nature, growing on their natural substrates. This is not always possible, however, especially during the winter months, when the tests are most likely to be made. Furthermore, the writer has found that spores obtained directly from nature generally vary more in their germinating ability than do those grown on artificial media. The spores from culture are thus more satisfactory to work with. Where the fungus is to be grown in culture, the choice of a suitable medium must be made. A suitable medium is one on which the fungus will produce an abundance of spores, and spores that will give maximum germination.

The maintenance of a high percentage of germination by spores from artificial culture following isolation from nature may present a problem. It was found, for example, that conidia of *Sclerotinia americana* obtained from rotting cherries gave a germination of about 94 per cent. As winter approached, the fungus was isolated and grown on potato-dextrose agar. At first the germination was very poor, conidia from the second transfer giving only 10 per cent of germination. Conidia from the third transfer germinated better, but it was not until the fourth transfer that the spores again gave their maximum germination, this time about 98 per cent — slightly higher than in nature. It has been found also, with certain fungi, that after many generations on artificial media the spores produced tend to lose their viability. An effort was therefore made to keep the number of generations to a minimum by always transferring from the oldest culture available. The fungus *Sclerotinia americana* has been grown continuously in culture for thirty months, new plantings being made about every week to obtain spores for germination tests, and yet only the fourteenth generation has been reached and the fungus still maintains its high germination ability of 98 per cent.

Age of culture

Although the age of the culture is not a definite indication of the age of the spores produced thereon, there is nevertheless a more or less well-defined correlation. The common practice has been to specify the age of the spores by the age of the culture at the time when the spores are taken for the test. In comparative tests the age of the spores should be known, and preferably they should be of the same age. Doran (1922: 316), in an interesting contribution to our knowledge of the factors influencing spore germination, has concisely stated, "Until the spore has attained a certain age or degree of maturity, it cannot germinate," and also (page 318 of same reference), "For all spores there is a maximum time limit, variable with the environmental conditions, beyond which the spore cannot germinate. The spore may be alive and too old to germinate or it may be dead." This definite time limit within which the spore will germinate should be borne in mind. Even within this limit there is a fairly well-defined period of optimum germination; and it is a point of major importance that as the age for optimum germination is passed the spore becomes more susceptible to the toxic effects of fungicides.

This relationship of spore age to toxic sensitivity in the case of 300-mesh dusting sulfur and *Sclerotinia americana* is well shown in figure 2, which has been compiled from the results of two experiments. The rapid drop in the sulfur-germination curves points to the extreme importance of the age factor and the necessity of knowing these relations when making fungicidal tests. A similar effect of copper-lime dust on the germination of *Macrosporium sarcinaeforme* spores is shown in table 1. Doubtless this factor applies also under field conditions, but this point cannot easily be proved.

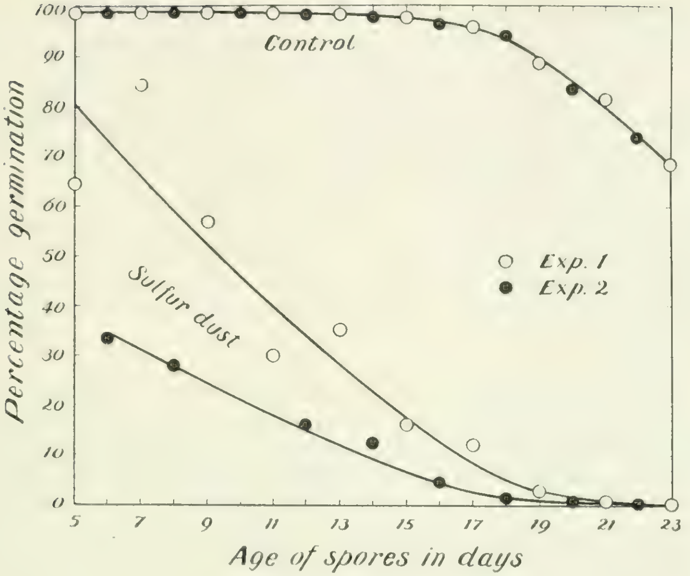

FIGURE 2. TOXICITY OF SULFUR DUST AS RELATED TO THE AGE OF THE SPORE: PERCENTAGE GERMINATION OF CONIDIA OF SCLEROTINIA AMERICANA

TABLE 1. RELATION OF SPORE AGE AND TOXICITY OF COPPER-LIME DUST, TO PERCENTAGE GERMINATION OF MACROSPORIUM SARCINAEFORME*

Age of spores, in days	Per cent germination	
	Control	Copper-lime
16	98.1	9.7
24	97.3	2.9
35	97.1	1.7
54	95.0	1.0
75	95.6	1.1
95	93.9	0.6

* Data from results obtained by Dr. J. G. Horsfall, formerly of this laboratory.

Kind of water

Among the external factors affecting germination, it is most logical to consider first the type of water best suited for the germination of the spores. In general, for this purpose there is distilled, tap, or rain water, the last including lake, stream, and spring waters. The last-named type of water will of necessity vary greatly. Although meteoric waters — that is, rain or dew — will be present under natural conditions, it is felt that they are not ideal for tests because of their great variation in composition. Distilled water would seem to be the most suitable, provided the spores will germinate well in it. Doran (1922) found that the distilled water which he used lacked aeration, and unless it was artificially aerated it reduced germination. Lack of aeration has not proved a serious obstacle in the writer's studies. All the spores that he tested required precipitated moisture for germination. In some cases, spores germinate equally well merely in a moist atmosphere (Doran, 1922). Certain types of spores are placed in suspension in water only with the greatest difficulty, and as yet no method has been devised to satisfactorily overcome this.

Concentration of spore suspension and oxygen relations

All workers have found that the presence of oxygen is essential for normal spore germination (Uppal, 1926). In all probability there is never an excess of air dissolved in the water, as far as the spore requirements are concerned. Hence the size and shape of the drops of water on the slides must be considered. It has generally been found that the flatter the drop, the better will be the germination. Also, the drops should all be of the same size in order that the oxygen relations and the possible staling effects may be constant. Finally, unless the drops are of the same size, surface and vapor-tension phenomena will result in the larger drops increasing in size at the expense of the smaller ones.

Doran (1922:332) noted " the injurious effect of the presence of fungous spores on the germination of the spores of other fungi." He attributed this to a competition for oxygen. He states: " When there is lack of sufficient oxygen for all, only those spores which require the least will germinate." Obviously, only one kind of spores should be present in the drop.

Because of the oxygen limitations and the staling effect (Wallace, Blodgett, and Hesler, 1911:173), there is a limit also to the number of spores that will germinate in a given drop of water. Hence there is an optimum spore concentration. As a general rule it may be said that the fewer the spores, the higher is the percentage of germination. However, if the number of spores is very much reduced, the probable error for germinations in a single drop will become unnecessarily high. Therefore, in making up spore suspensions in water the object should be to obtain as high a concentration of spores as is possible without unduly reducing the germination.

The relations of spore concentrations to germination for *Botrytis allii* are shown in table 2. In this, as in all succeeding tables, the number of spores is based on a low-power microscopic field of 1575 microns diameter. Here, as can be seen, it is not advisable to have a spore concentration greater than 50 spores per field.

TABLE 2. Spore-Concentration and Germination Relations for Botrytis allii in Water

(80 per cent sterile lake water, 20 per cent tap water, 21° C.)

Number of spores	Per cent germination
20– 50	92.5
51– 75	88.4
76–100	85.4
101–125	82.5
126–150	72.3
151–200	67.7
201–300	48.5

The relations of spore concentrations to the kind of water, for the conidia of *Botrytis allii* and *Cephalothecium roseum*, respectively, are shown in tables 3 and 4. For *Botrytis allii* lake water is seen to be the most suit-

TABLE 3. Spore-Concentration and Water Relations for Botrytis allii

(Percentage germination at 21° C.)

Number of spores	Per cent germination		
	In lake water	In tap water	In distilled water
15– 25	97.0	92.2	94.5
26– 50	95.5	72.2	61.7
51– 75	93.5	71.5	43.4
76–100	91.2	......	......
101–150	84.1	......	4.3

TABLE 4. Spore-Concentration and Water Relations for Cephalothecium roseum

(Percentage germination at 21° C.)

Number of spores	Per cent germination		
	In rain water	In tap water	In distilled water
20– 50	50.3	30.7	62.7
51– 75	36.6	24.6	60.0
76–100	12.7	23.2	56.1
101–150	10.7	22.8	34.2
151–200	8.2	19.3	26.3
201–300	......	......	18.0
301–400	......	......	9.2

able, and for *Cephalothecium roseum* distilled water. With each in its best-suited water, germination rapidly decreases when a spore concentration of 50 is exceeded. In no case within the experience of the author has the optimum spore concentration exceeded approximately 50 spores in a microscopic field of 1575 microns diameter. Indications are that the germination will be slightly increased as the number of spores is reduced from 50; the difference, however, is not significant. As a general rule, a spore concentration of 15 to 50 has been found the most satisfactory.

Temperature

The temperature relations for spore germination are probably better known than is any other factor. The optimum temperature for the germination of the spores of any fungus should be ascertained before laboratory tests are attempted. In table 5 is shown the effect of temperature and spore concentration upon spore germination for the conidia of *Sclerotinia americana*, and in table 6 the effect of temperature and kind of water for conidia of *Glomerella cingulata*.

TABLE 5. TEMPERATURE AND SPORE-CONCENTRATION RELATIONS FOR CONIDIA OF SCLEROTINIA AMERICANA

(Fifth transfer, 8 days old, in distilled water. Percentage germination)

Temperature (C.)	Spore concentration			
	20–50	51–100	101–200	201–400
	Per cent germination			
12.0°	42.2	11.8	0.0	0.0
15.5°	59.0	29.4	0.0	0.0
17.5°	93.9	50.5	11.1	0.4
21.0°	98.1	77.8	36.7	0.8
23.0°	99.0	90.5	57.1	7.1
25.5°	99.0	91.8	55.0	9.5
27.0°	96.5	90.0	31.7	1.0
30.0°	80.5	20.0	5.1	0.0

TABLE 6. TEMPERATURE AND WATER RELATIONS FOR CONIDIA OF GLOMERELLA CINGULATA

(Percentage germination)

Temperature (C.)	Per cent germination		
	In rain water	In tap water	In distilled water
12°	28.3	32.2	23.4
15°	38.5	31.4	51.5
18°	61.6	55.5	67.2
21°	67.3	76.3	69.0
24°	64.1	84.7	75.6

These tables show, as do the preceding ones, that, where two factors are varied, as the optimum of the one is reached differences due to the other tend to be minimized. Thus, for example, in the determination of the optimum temperature it might be well to vary another factor also in order that the temperature differences may be shown the more clearly.

The optimum temperature for spore production may not necessarily be the same as that for spore germination. An abundance of spores is essential, and it may therefore be necessary to maintain the cultures at a temperature for spore production which is different from that for optimum spore germination. Limited tests here seem to indicate this for conidia of *Venturia inaequalis.*

Light

None of the fungi tested exhibited any preferential light requirements. This appears to agree with the results of most other investigators (Doran, 1922).

Time

Since the time limits for the germination of spores vary greatly, it will be necessary to determine this period for each fungus. If the test is discontinued too soon, not all the spores capable of germinating will have done so; if the test is continued too long, the spores may have germinated to such an extent as to form an indistinguishable tangle, not to speak of the possibility of the drop's evaporating. Of course, in nature the optimum conditions for germination may be present for such a short time that but few spores may ever germinate successfully. In spite of this, since fungicidal tests are chiefly comparative in character, it is advisable to allow sufficient time for optimum germination. Doran (1922) has compiled from the literature an interesting list of the lengths of time required for representative fungus spores to germinate.

APPLICATION OF THE FUNGICIDE

It is very essential that care be exercised in the application of the fungicide to the slides. For applying liquid fungicides a DeVilbiss atomizer, "Atlas Spray No. 29," has been found very satisfactory and will deliver the fine, uniform, mist-like spray desired. The dry fungicides, or dusts, are applied by means of test-tube dusters, one of which is illustrated in figure 1, a (page 11). These were devised by Dr. F. M. Blodgett, of this laboratory, and have long been in use here. They are now widely employed in other laboratories as well (Lee and Martin, 1927:316). They are very simple in construction, being made of a heavy-walled six-inch test tube, a two-hole rubber stopper, two pieces of bent glass tubing, and a rubber atomizer bulb. Glass tubing of 5 millimeters outside diameter and 3 millimeters bore is very suitable. Two pieces are bent in a gradual L curve. The shorter delivery piece is placed to project just below the rubber stopper, while the longer piece, to which the atomizer bulb is attached, is just immersed in the dust. For best results the test tube should contain about an inch of dust. A separate test-tube duster must be used for each dust. A thorough washing is of course essential before the tube is used for another dust. If the dusters are to be used at frequent intervals, the dust may safely be left in the tube.

If consistent results are to be obtained, it is imperative that an even layer of spray or dust be applied to the slides. If too much spray is applied, it will tend to coalesce in large drops. A fine, even mist is the most satisfactory. It is more difficult to get an even layer of dust. Large particles may cause trouble, though oftentimes these may be blown off without disturbing the finer dust. The tendency is to apply too much rather than too little dust. In nature the average leaf seldom receives a heavy coating of dust or spray.

The general impression is that the greater quantity of fungicide used, the greater is the fungicidal efficiency. Accordingly some limited tests were carried out to study this point. Light, medium, and heavy applications of Niagara 300-mesh sulfur, Niagara Kolodust, and lime-sulfur 1:40, were made. The spores used were conidia of *Sclerotinia americana*. The results are shown in table 7.

TABLE 7. Results of Tests to Determine the Relation between Quantity and Efficiency of a Fungicide

(Percentage germination of *Sclerotinia americana* conidia at 24° C. in distilled water)

Fungicide	Application	Per cent germination		
		Experiment 1*	Experiment 2*	Experiment 3*
Niagara 300-mesh sulfur	Light..........	49.4 B	92.4 B+	76.2 B+
	Medium........	35.8 C	78.3 C	57.8 B-
	Heavy.........	36.3 C	77.2 C	37.5 C
Niagara Kolodust	Light..........	24.6 C	70.0 C-	10.0 D
	Medium........	1.0 D	17.3 D	1.0 D
	Heavy.........	0.3 D	7.5 D	0.0
Lime-sulfur 1:40	Light..........	0.0	1.8 D	....
	Medium........	0.0	Trace D	....
	Heavy.........	0.0	Trace D	....
Control	....	94.7 A	98.6 A	96.6 A
Age of spores, in days.......	....	15	13	17

* See page 21 for interpretation of letters.

Similar results were obtained with the uredospores of *Uromyces hybridii*. These are given in table 8.

TABLE 8. RELATION OF QUANTITY AND EFFICIENCY OF SULFUR DUST TO GERMINATION OF UROMYCES HYBRIDII UREDOSPORES*

(Distilled water at 21° C.)

Fungicide	Application	Per cent germination
Niagara 300-mesh sulfur	Light	85.1
	Heavy	92.8
Niagara Kolodust	Light	63.3
	Heavy	58.2
Control		92.6

* Data from results obtained by Dr. J. G. Horsfall, formerly of this laboratory.

The results of these experiments, though exceedingly limited, would seem to indicate that, provided a certain minimum of sulfur is applied, increasing the amount beyond this will not appreciably increase the fungicidal efficiency.

RECORDING RESULTS

As has already been mentioned, the test should not be concluded and the results taken until all viable spores have germinated. There are three common ways of expressing the toxicity of a fungicide: one can record the percentage of germination, can record the character of the germ tubes, or can attempt to grow the treated spores as was done by Lee and Martin (1927) and by Young and Cooper (1917). There are objections to all three. With this method of testing fungicides under asterile conditions, the last-named practice is impossible. Extended measurements of germ tubes involve much time and labor and are of doubtful value since the tests are only relative. The method first employed by the writer was merely to record the percentage of germinated spores. This was later supplemented by observations on the length and the character of the germ tubes. The germ tubes are provisionally classed into four types, A, B, C, and D, of which A is the largest and most vigorous, normally corresponding to those in the check, and D is the poorest. In general, D type includes spores that have just germinated and all up to those that have produced a germ tube approximately 25μ long; C type includes those with germ tubes 25 to 100μ long; B type, those with germ tubes 100 to 200μ long; and A type, those with germ tubes 200μ in length and longer. In addition, the signs + and − may be employed in each class. From the observations of Kightlinger (1925), it is probable that spores of D type, and probably also of C type, would be incapable of producing infection. The spores are not classified individually, but as a whole into one type for each microscopic field.

The drops of spore suspensions cannot all be made exactly the same size, nor will they encounter exactly the same conditions. Therefore, in order to avoid magnifying small differences, the general rule has been never to count more than one microscopic field of spores in a single drop. Occasionally, because of a limited number of spores it may seem advisable to count more than one field to a drop, but in this case the same number of fields should be counted in each drop. Assuming 25 spores in one field, and one moist chamber to a test, 400 spores will have been counted for one test. If possible, it is more convenient to use the low-power objective in observing the spores. In this case a cover slip will not be required. For convenience in counting, the field may be divided into quadrants. To accomplish this, a cover slip on which thin lines are drawn in ink at right angles to and bisecting each other, is placed on the ocular diaphragm of the microscope. To facilitate and expedite the counting, two Veeder Hand Tally Counters are used, one to record germinated and the other ungerminated spores. Absolute germination percentages should be recorded, and not relative germinations where all controls are raised to 100 and the other readings elevated in proportion as has been done by some workers. This latter method ignores the disproportionate effect of treatment on spores of lower viability, discussed earlier, and masks the variation between tests so that true comparisons cannot be made.

It is impossible to completely control all the conditions surrounding the laboratory testing of fungicides, especially in view of the variability and the sensitiveness of most spores (Duggar, 1901). An average of 400 or 500 spores to a test will tend to give, in the germination results, a somewhat high probable error. A repetition of tests is therefore very necessary. This may be accomplished by performing duplicate or triplicate moist-chamber tests, or by repeating the experiment. One should not expect to duplicate the figures of a former experiment. In table 9

TABLE 9. Results of Sulfur Tests on Conidia of Venturia inaequalis
(Percentage germination at 21° C. in distilled water)

Treatment	Experiment 1	Experiment 2	Experiment 3	Average
Niagara 300-mesh sulfur	18.3	39.5	44.6	34.1
Banks' colloidal sulfur diluted with Bentonite: 0.3 per cent sulfur	18.4	34.5	49.2	34.0
Banks' colloidal sulfur diluted with Bentonite: 1.0 per cent sulfur	13.0	27.5	49.2	29.9
Banks' colloidal sulfur diluted with Bentonite: 10 per cent sulfur	....	9.6	24.0	16.8
Niagara Kolodust (colloidal sulfur 10 per cent; dusting sulfur 90 per cent)	9.2	2.8	23.8	11.9
Bentonite	39.2	44.6	64.0	49.3
Control	47.2	50.6	69.5	55.8

are listed the results of three experiments on the effect of sulfur dust on the conidia of *Venturia inaequalis*. This gives an indication of the variation obtained between experiments performed at different times.

SUMMARY

1. The practicability, advantages, and limitations of laboratory tests for fungicides are discussed. It is believed that such tests are reliable and significant.

2. A method for testing developed at the Laboratory of Plant Pathology at Cornell University is described. Briefly, the technic is as follows: Glass slides are sprayed or dusted with the fungicide to be tested, and are then placed in inverted moist chambers. The spores of the fungus are suspended in water, and drops of this suspension are placed on the sprayed or dusted slides and on the check slides. The moist chamber is sealed with water, giving a highly saturated atmosphere in which the spores are left to germinate at the desired temperature. After a given time, counts are taken to determine the percentage and the type of germination.

3. The necessity for standardization of the conditions for germination and the factors relative thereto, such as the source and age of spores, type of water, spore-suspension concentration, oxygen relations, temperature, light, and time, is discussed.

4. Details concerning methods of applying the fungicides and the recording of data are presented.

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STUDIES ON FUNGICIDES

III. THE SOLVENT ACTION OF SPORE EXCRETIONS AND OTHER AGENCIES ON PROTECTIVE COPPER FUNGICIDES[1]

S. E. A. McCallan

In the first paper of this series it was pointed out that protective fungicides, or protectants, are in general insoluble in water. Kahlenberg and True (1896) have shown that the toxicity of copper salts is due to the ions in solution. Therefore, in the case of copper protectants it may generally be assumed that copper ions effect the toxic action and hence account for the protective efficiency of the fungicide. The question then arises as to the manner in which the copper is rendered soluble, since presumably it cannot exercise its toxic properties while in the insoluble state. It is thus evident that, though the copper protectant must be essentially insoluble in water, yet in the presence of pathogenic spores it must become soluble to a sufficient extent to kill the fungus, or at least to prevent infection of the suscept.

This paper sets forth the results of some studies on the manner in which the soluble copper is liberated from the nominally insoluble copper compounds. No attempt has been made to study the actual toxic action, or the manner in which the copper kills.

Most investigations relating to the nature of the fungicidal action, or, more specifically, the liberation of the copper from copper protectants, have dealt with bordeaux mixture. This is to be expected, as bordeaux mixture is our oldest and most important copper protectant. The studies here described have likewise dealt with this protectant, and also with copper-lime dust.

The chemical and the physical nature of the compound or compounds resulting from the reaction of copper sulfate and calcium hydroxide to form bordeaux mixture, are of great complexity and variability. Despite the extensive investigations on this subject, there is yet much to be clarified. The first workers, Millardet and Gayon (1885, 1887 b), and other early workers, such as Chester (1890), considered the reaction simple, holding the active fungicidal constituent formed to be cupric hydroxide. Sostegni (1890), Fairchild (1894), and Swingle (1896) state that the copper is present as a basic sulfate in addition to the hydrate. More recent workers — Pickering (1907, 1909), Bedford and Pickering (1910), and

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Butler (1914, 1923) — as the result of their investigations are inclined to the view that various basic sulfates of copper are formed, in addition, of course, to the calcium compounds. The actual basic copper sulfates formed are said to depend largely on the ratio of copper sulfate to limewater or milk of lime. However, Bell and Taber (1907), the only investigators to apply the phase rule to this study, claim that the composition is constant provided a fixed temperature is maintained, and that (page 636 of reference cited) the toxic action of the copper must " be attributed either to the solid copper hydroxide (and it is difficult to see how this could act unless in solution), or else to the very dilute copper solution."

A most important feature is the colloidal nature of bordeaux mixture (Swingle, 1896, and Lutman, 1912, 1916), for it is upon the peculiar blue precipitation membranes that the protective value of this compound rests.

A good discussion of the nature of copper-lime dust, an approximate equivalent of bordeaux mixture, is given by Boyd (1926). Holland and Gilligan (1928) have presented a general classification of copper fungicides.

HISTORICAL REVIEW

A review of the very extensive literature on the liberation of the copper from bordeaux mixture reveals that, in the main, three hypotheses have been advanced to explain the freeing of the copper from the colloidal membranes. These hypotheses are stated by Barker and Gimingham (1911:76) thus:

> 1. That copper is brought into solution by atmospheric agencies — more especially by the action of the carbon dioxide of the air, *i. e.* a purely chemical explanation.
> 2. That the leaves, on to which the mixture is sprayed, exert a solvent action on the compounds, *i. e.* an action of the host plant.
> 3. That the fungus itself is responsible for the production of the soluble copper by which it is finally poisoned, *i. e.* an action of the fungus.

It is not the intention to present here an exhaustive review of the literature. In general, only those articles containing original experimental evidence in support of these respective hypotheses are summarized. More recent articles are discussed in somewhat more detail.

ATMOSPHERIC AGENCIES

The hypothesis assuming that atmospheric agencies free the copper from bordeaux mixture, has, without doubt, received the most attention, and perhaps at the present time is the most generally accepted. This hypothesis had its origin in the very development of bordeaux mixture itself, for in 1887 Millardet and Gayon (1887 a:161) stated (translation of original): " In the first place, it happens that water from rain and dew, in consequence of its carbonic acid and carbonate of ammonia, dissolves a sufficient amount of copper hydroxide which is on the leaves, either to completely prevent the germination of conidia of Peronospora in this water, or at least so that it cannot take place in a normal manner." Again, to quote these investigators (1887 b:700, translation): "We have proved that the copper hydroxide contained in a drop of the mixture is soluble in rain and dew only when the lime which in the actual mixture is found in enormous excess is completely neutralized, especially by the carbon dioxide of the atmosphere." Millardet and Gayon based this conclusion on an experiment in which they prepared five types of bordeaux mixture, ranging from copper sulfate very slightly in excess, to that containing lime eight times in excess, of neutralization. These mixtures were exposed to the rains. The time of the first appearance of copper in the solution varied from five to eighteen days. Clark (1902:43), Crandall (1909:225), Bedford and Pickering (1910:72), Barker and Gimingham (1911:94), and Lutman (1916:17) all criticize this conclusion, and point out that there is no delay in the fungicidal action of the copper but that it is effective from the time of application. Clark (1902:43), in fact, thinks this should be "sufficient to have set aside the hypothesis which accounted for the solution of the copper of the bordeaux mixture by atmospheric conditions."

Sostegni (1890), experimenting with bordeaux mixture, passed carbonic-acid gas through the copper hydrate and basic sulfates and succeeded in bringing copper into solution. He therefore assumes that the CO_2 of the atmosphere has a solvent action on the precipitated copper compound. These conclusions are accepted by Lodeman (1892).

Fairchild (1894) is inclined to the view that the copper compounds are slowly dissolved by the rains and dews. He also was able to obtain soluble copper on the addition of CO_2.

Though performing no experiments himself, Swingle (1896) suggests various possibilities. He mentions the possible rôle of atmospheric agencies, but says (pages 19-20 of reference cited): "It seems scarcely possible that the minute quantities of CO_2 contained in rain water or dew alone could dissolve enough copper to poison any but the most delicate plants. Probably, as claimed by Gayon, the copper hydroxide present is slightly soluble in pure water."

Bain (1902) seems to have shown conclusively that the injurious action of soluble copper on peach leaves, resulting from spraying with bordeaux mixture, copper hydroxide, and dicupric hydroxide ($Cu_3O_2(OH)_2$), is most pronounced when the plants have been exposed to the rains and dews. This injury was found to be further accentuated in the presence of sunlight.

Ruhland (1903), studying the effect of plant excretions on the solution of basic copper, found that rain water containing CO_2 and other substances from the air could dissolve very small amounts of copper. However, he did not consider that this amount of copper exercised any fungicidal action, except possibly as a secondary effect in the case of very sensitive fungi, though he conceded that it probably was a factor in spray injury.

Schander (1904) does not believe that atmospheric agencies are ordinarily very effective. He placed various bordeaux mixtures in crystallizing dishes, one lot being exposed to the sun and the other kept in the shade. Both lots were periodically moistened with rain water. Soluble copper appeared in neutral bordeaux after 23 days, but in alkaline bordeaux not until 42 to 56 days. Butler (1914:142), however, questions the trustworthiness of Schander's test for soluble copper.

Crandall (1909) performed extensive field tests with apple trees sprayed with bordeaux mixture. The trees were either exposed to the rain and dew, or wetted with cistern water, carbonated water, or distilled water, and the drip was collected and analyzed for soluble copper and alkalinity. The freshly made bordeaux mixture contained no free copper. The drip waters did not give a neutral reaction until late in the summer, but soluble copper was present from the first and continued as long as any bordeaux was present, thus attesting to the slow rate of solubility of the copper from bordeaux mixture. Rain water and carbonated water were equally effective in bringing copper into solution. Cistern and distilled water were only about half as effective. The amount of soluble copper present was from a trace to 1 part in 11,000 parts of water. Excess lime diminished the extent of the solubility of the copper, but did not entirely prevent it. Limited laboratory experiments, however, gave contradictory results. Bordeaux mixture in crystallizing dishes, either dry or intermittently wet with cistern water, gave no soluble copper at the end of 65 days. Nor did sprayed trees, also either dry or frequently moistened with cistern water, show any copper in the wash waters at the end of 54 days. Crandall (1909:226) concludes "that meteoric waters possess solvent powers that do not belong to the water artificially applied." He assumes that the CO_2 is the chief dissolving agent, but that it is probably supplemented by other agencies, possibly ammonium compounds, nitrates, and nitrites.

Pickering (1907, 1909), and Bedford and Pickering (1910), undertook numerous laboratory experiments in an effort to demonstrate the solvent action of the CO_2 of the atmosphere. Carbon dioxide was passed through suspensions of the various basic sulfates of copper, the length of time required and the amount of copper brought into solution being noted. Copper from the Woburn bordeaux ($4CuO, SO_3$) was found to be immediately soluble, while in the case of ordinary bordeaux ($10CuO, SO_3, 3CaO$) there was a considerable delay. Pickering states that this delay is due to the presence of excess lime. The CO_2 carbonates the lime most readily, though it acts to some extent on the basic sulfate of copper, producing ordinary copper sulfate. The excess lime still present, however, reconverts this soluble copper sulfate to the basic sulfate. This cycle continues, finally resulting in only a part of the total copper being brought into solution, and then not until nearly all of the excess lime has been carbonated.

In nature there is no appreciable delay in the fungicidal action of bordeaux mixture. Pickering explains this apparent contradiction by stating that in the laboratory the basic sulfates were constantly wet and any soluble copper found would be immediately precipitated by the limewater. In nature the bordeaux mixture dries, and the particles of basic sulfate and of lime become separated from each other. Hence, when the former is attacked by the CO_2 there may be no lime particles sufficiently near to decompose the copper sulfate formed. In view of the fact that fungicidal protection is usually exerted only when the spores are on a wet surface, this explanation seems hardly sufficient. Pickering considers this theory established by a simple laboratory experiment, in which filter paper is moistened with potassium-ferrocyanide solution and dried. When a drop of bordeaux mixture is placed on the paper, there is no reaction as long as it is wet. However, immediately on drying, the lime particles no longer have access to the particles of basic sulfate, and soluble copper appears as is shown by the formation of red copper ferrocyanide.

Gimingham (1911) also passed CO_2 through suspensions of the various basic sulfates similar to those used by Pickering (1907, 1909) and by Bedford and Pickering (1910). In all cases the resulting soluble copper was immediately reprecipitated on the removal of the CO_2. This led Gimingham to believe that the soluble copper was probably in the form of a carbonate dissolved in the CO_2 solution. When the action of the CO_2 was prolonged, the soluble copper did not easily reprecipitate. The action of ordinary air brought little or no copper into solution. Gimingham concluded that only in the presence of a large excess of CO_2, such as could hardly occur out-of-doors, is an appreciable amount of copper brought into solution, and that " it appears impossible to assign the fungicidal action to copper sulphate liberated by *atmospheric* carbon dioxide " (page 75 of reference cited).

Barker and Gimingham (1911:86) state: " The fungicidal action of the bordeaux compound was evidently not in any way dependent upon the presence of carbon dioxide." This conclusion is based on an experiment in which conidia of *Nectria ditissima* and *Sclerotinia fructigena* were sown on a fresh precipitate of no-excess-lime bordeaux ($10CuO, SO_3$). In the absence of CO_2, there was very poor germination and any feeble growth was soon halted. The response of the controls, exposed to the air, was the same.

Pickering attempts in a later paper (1912) to refute the criticisms of Gimingham (1911) and of Barker and Gimingham (1911), and presents new evidence. Air was passed through suspensions of the various basic sulfates and copper carbonate in which bright iron rods were placed. According to Pickering, the loss in weight of the iron rods should give the amount of soluble copper abstracted from the suspension, and this is a much more delicate test than that by the potassium ferrocyanide. Soluble copper was detected, but in the case of bordeaux mixture there was none until all the free lime had been carbonated. The respective amounts of copper removed after three hours were: in the case of $4CuO, SO_3$ – 7.3 per cent; $10CuO, SO_3$ – 4.2 per cent; $10CuO, SO_3, 3CaO$ – 2.3 per cent; and $2CuO, CO_2$ – 1.0 per cent. In another experiment, air containing various percentages of CO_2 was passed through a suspension of $10CuO, SO_3$ for three hours. The relative amounts of copper brought into solution were: 0 per cent CO_2 – 109; 0.03 per cent CO_2 – 100; 10 per cent CO_2 – 58; and CO_2 only – 834. The fact that, with the exception of an atmosphere of CO_2 only, the solvent action is correspondingly greater as the amount of CO_2 in the air is reduced, reflects seriously on the possible rôle of atmospheric CO_2 in bringing copper into solution. Pickering (1912: 274), however, maintains that the fungicide would be " bathed in an atmosphere of the carbon dioxide evolved from the leaves themselves."

These conclusions of Pickering (1912) were further criticized by Barker and Gimingham (1914 b). These workers point out that the amount of CO_2 present in the atmosphere would retard rather than promote the solution of copper, according to Pickering's own results. Also, they note that the CO_2 from the leaves is rapidly dispersed. This assertion is based on the results of an experiment in which a large strawberry plant was covered with Woburn bordeaux paste ($10CuO, SO_3$) and " placed in a bowl of water under a bell-jar in order to keep the atmosphere saturated. Under these conditions the action of any CO_2 respired by the leaves would presumably be at a maximum " (page 221 of reference cited). After three days no copper could be detected in the condensed water on the leaves. The potassium-ferrocyanide test was employed.

Groth (1910), studying bordeaux injury on peaches, was able to render the copper soluble with distilled water and tap water as well as with rain water. He found also that the lower surface of the leaf was apparently more sensitive to injury than the upper. Peach leaves have no stomata in the upper epidermis. In the laboratory, injury occurred only when there was a covering of water on the leaves, a high relative humidity, and some cloudy weather. Groth therefore offers the theory (page 19 of reference cited): " An excess of CO_2 evolved in the shade, passes into the water standing on the leaf during wet weather. CO_2 is dissolved, and the carbonated water thus formed dissolves some of the copper."

Butler (1914) carefully reviews the literature relative to the action of CO_2 on bordeaux mixture. He appears, however, to have performed but few experiments himself. In one case he found that carbonated bordeaux mixture containing 0.5 per cent of cupric sulfate was not toxic to spores of *Phytophthora infestans* or of *Plasmopara viticola* in tap water, but that when distilled water was used, regardless of the ratio of cupric to calcic oxide, the mixtures were toxic at 0.0625 per cent. This difference, he explains, is due to the fact that tap water contains calcic bicarbonate in solution, and " calcic bicarbonate is a precipitant of soluble copper in the electro-positive condition " (page 139 of reference cited); hence, because of this, the tap water is unable to dissolve the copper in bordeaux mixture. Butler therefore concludes, from this experiment, that bordeaux mixtures after carbonization are slightly soluble in pure water, and that they will dissolve readily in water containing CO_2 in solution — the latter presumably a conclusion drawn from the literature. Finally, he holds that these mixtures are soluble also in water containing ammonic salts in solution. No experimental proof is presented for this last conclusion.

Pantanelli (1920), from his investigations, is inclined to the view that copper sprays are efficient for long periods, as the hydroxide is slowly acted upon by the CO_2 and rendered less basic and more soluble.

Cerasoli (1926) advances the hypothesis that many of the components of bordeaux mixture, such as the basic sulfates of copper, are hydrolyzed by water to form gelatinous copper hydrate. The copper hydrate dissociates very slightly, giving copper and hydroxyl ions. Exposed on the leaf, the carbonic acid derived from the CO_2 of the atmosphere and of plant respiration furnishes hydrogen ions. These hydrogen ions combine with the hydroxyl ions of the base to form water which is practically undissociated. The product of the copper and hydroxyl ions then becomes too small, and the equilibrium is destroyed by a further dissociation of the copper hydrates. This production of copper and hydroxyl ions will continue as long as there remains any undecomposed copper hydrate. In such a manner the sprayed plant parts are coated with an infinitesimal amount of soluble copper, which is sufficient to inhibit the germination of fungus spores. Cerasoli offers no experimental evidence.

Schmidt (1924) reviews the literature briefly, and states that of some six theories only two can now be considered as probable; namely, that of Millardet on the action of atmospheric CO_2, and that of the solvent action of the spores as advanced by Ruhland (1903) and others. With these two hypotheses in mind, Schmidt undertook various experiments. Excess-lime bordeaux mixture was made, potassium ferrocyanide was added, and leaves of apple, pear, and grape were sprayed. Only after a lapse of time did the red color appear to show the presence of soluble copper. Similar results were obtained on filter paper. A little potassium ferrocyanide added to copper hydroxide in distilled water, and the whole placed in a dish covered with paraffin, showed no color after a month, whereas a similar mixture in an open dish showed the red color after one day.

Botrytis spores were placed by Schmidt in distilled water in an open dish and allowed to germinate for 48 hours. A control without spores was exposed for 4 hours. To each there was then added a little copper hydroxide, and after 30 minutes a test for soluble copper was made with potassium ferrocyanide. The test was positive and of equal intensity for both.

In another experiment Schmidt allowed distilled water to stand on copper hydroxide for four weeks, and then filtered. Botrytis spores were placed in the filtrate, and also on the precipitate, to which fresh distilled water had been added. These were each divided into two series; to one a little sugar was added, and to the other none. The results after three days were good germination in the precipitate, both with and without sugar, about one-third germination in the filtrate with sugar, and no germination in the filtrate without sugar. From this Schmidt concludes that the four-weeks-old filtrate was more toxic than the precipitate suspension in fresh water, and that this difference was due to the time of standing exposed to the atmosphere.

Schmidt also prepared four sets of tubes, in each of which was placed a suspension of copper hydroxide in distilled water. Trichothecium spores were sown in two of the sets, and the ends of the tubes of one set were sealed with paraffin. One of the two remaining sets was likewise sealed. After standing thus for 48 hours, potassium ferrocyanide was added to all and the color reaction was observed in 5 hours. The two sealed tubes gave a very slight reaction for soluble copper, while the unsealed tubes gave a very decided reaction, thus showing the presence of soluble copper. There was no apparent difference in the tubes with or without spores. This test was repeated with bordeaux mixture and spores of Botrytis and Aspergillus. The results were more marked this time, in that there was no reaction at all in the sealed tubes.

A similar type of experiment was set up by Schmidt, in which washed copper hydroxide was placed in watch glasses in distilled water. Half the glasses were placed in a desiccator over potassium hydroxide. The other half were left exposed to the air. To half of each of these sets, Botrytis spores were added and allowed to germinate for 48 hours, when potassium ferrocyanide was added to all. After another period of 48 hours there was only a very slight positive test for copper in the glasses in the desiccator, but after only 5 hours there was a very positive test in the exposed dishes. Schmidt states that the spores in all the dishes gave about 50 per cent germination. This appears very inconsistent with the fact that he found such marked differences in the amounts of soluble copper in the two sets.

In still another experiment, designed more or less to duplicate natural conditions, Schmidt stretched collodion membranes over one side of glass rings. Copper hydroxide or bordeaux mixture in agar was placed on the underside of the membranes. The rings were divided into two lots, and to one of the lots sugar was added. On the outside of the membranes, spores of Botrytis, Monilia, or Fusicladium were sown, along with the fungicide. With the copper hydroxide, all the spores germinated freely while in contact with the copper-hydroxide particles, either with or without sugar. With the bordeaux mixture, however, with or without sugar, there was restricted germination, though any germination or subsequent growth was slightly better where sugar was added. This experiment was further modified and set up in six series. Series 1 and 2 consisted of watch glasses, series 1 containing 2 per cent of copper hydroxide, and series 2 containing 2 per cent of bordeaux mixture, in distilled water. A trace of sugar was added to each. Series 3 and 4 were glass slides, sprayed, in the case of series 3 with 2 per cent of copper hydroxide, and in the case of series 4 with 2 per cent of bordeaux mixture. These were allowed to dry in the air. Series 5 and 6 consisted of glass rings over which the collodion membrane was stretched. In series 5 a little copper hydroxide was rubbed over the surface of the membrane, and on the inside a paste of 2 per cent of copper hydroxide with a trace of sugar was added. Series 6 was treated in the same way except that bordeaux mixture was used instead of copper hydroxide. Spores of Botrytis were sown on all six, the whole series was placed in moist chambers, and the spores were allowed to germinate for six days. The results were as follows: series 1,100 per cent germination: series 2, 20 per cent; series 3, 100 per cent; series 4, 2 per cent; series 5, 100 per cent; series 6, 0 per cent; control, 100 per cent.

In a final test, Schmidt found that Botrytis and Aspergillus germinated only very poorly in limewater. However, limewater apparently did not hinder the germination of Trichothecium spores.

Schmidt concludes from his studies that there is no exosmosis of substances from the spores which promotes the solution of bordeaux mixture or of copper hydroxide. He concludes also that bordeaux mixture is more toxic than copper hydroxide, and that the fungicidal action of bordeaux mixture is due to the combined influence of the copper brought into solution by the carbonic acid of the atmosphere, and the repressive alkalinity of the mixture.

Schmidt did not demonstrate the repressive alkalinity of bordeaux mixture toward all his spores. Apparently, he assumes that the copper of bordeaux mixture is in the form of copper hydroxide. The copper hydroxide employed was very evidently soluble in distilled water to a very appreciable extent, since Schmidt used the potassium-ferrocyanide test, which is not very delicate. In the two last-described experiments a possible solvent action by the spores toward the bordeaux mixture is entirely possible. Schmidt's failure, in his first experiment, to obtain soluble copper from the sprayed bordeaux, is not in entire accord with his general conclusion, and also points to a delay in fungicidal action, which has been definitely established not to be the case.

In summarizing the literature on the action of atmospheric agencies, it should be emphasized that a great many of the experiments were performed with freshly made bordeaux mixture or other copper suspensions before they had been dried, as is the case when applied to plants. This evidence, therefore, can have no conclusive bearing on the liberation of copper from fungicides after they have been dried on the plant. Nor should the action of excessive quantities of CO_2 be taken as a criterion for judging the probable action of the very small quantities present in the atmosphere. There seems to be convincing evidence that meteoric waters possess a solvent action not exhibited by distilled water. Whether this difference is due solely to the CO_2 content has not been demonstrated. Under field conditions, with the plants exposed to the rain and dew, copper appears to be brought but slowly into solution from " insoluble " copper protectants.

SUSCEPT EXCRETIONS

The hypothesis that excretions from the sprayed plant (the suscept) exert a solvent action on the insoluble copper protectants, has received but little attention. From the mass of literature on injury and the physiological relations of bordeaux mixture and the suscept, there may be gathered a little direct experimental evidence relative to the solvent action of suscept excretions. The author makes no claim to having thoroughly reviewed this literature.

Swingle (1896:20) states: " It is possible that the drops of rain or dew standing for some time on the leaves or other parts of plants may absorb from superficial cells sugar or other substances that may serve to increase the solubility of the copper." Some observations of previous writers which led Swingle to suggest this hypothesis are presented.

Clark (1902) applied a spray of excess-lime bordeaux mixture to a peach tree. Following this, the tree was sprayed several times with " pure " water, to give the effect of dew. On the following day, water collected from the leaves showed the presence of a slight amount of soluble copper. Branches containing some of these leaves were placed in distilled water, and after some hours soluble copper was detected. Clark believes that there is an exosmosis of some of the contents of the cell sap, which, coming in contact with the bordeaux adhering to the leaf surface, cause more or less copper to become soluble. One is hardly justified, however, in drawing such a general conclusion from a limited experiment.

Bain (1902), studying bordeaux injury to peaches, performed a few experiments relative to the solvent excretion from leaves. He found that the gum secretion from peach leaves did not increase the injury. By the use of blue litmus solution no acid excretion could be demonstrated from the leaves. Peach leaves, paraffined glass slides, and plain glass slides were sprayed with copper hydroxide, and were then placed in the sunlight and the water allowed to evaporate. Following this, the surfaces were kept continuously wet with distilled water for several hours. This water from the treated surfaces was then filtered, and tests were made with cells of Spirogyra to detect soluble copper. The percentages of cells killed were, respectively, 88, 24, and 51, from which Bain (1902:93) concluded that " there is *something* escaping from the peach leaf which has a solvent action on copper hydroxide." Bain did not, however, give the percentages of cells killed in his controls, and in a preliminary experiment he found that the filtrate from a stock suspension of copper hydroxide was equally as toxic as that from the sprayed peach leaves. He assumes, in common with the earlier workers, that the copper of bordeaux mixture exists in the form of copper hydroxide.

Schander (1904), also studing bordeaux injury, discovered that the leaves of Fuchsia and Oenothera excrete acids which apparently can dissolve small amounts of copper hydroxide. In the case of *Phaseolus multiflorus*, the leaf excretions reacted alkaline and Schander was unable to demonstrate a solvent action.

Ruhland (1903), as a result of his investigations, was unable to substantiate the hypothesis of suscept excretions. Studying those plants especially susceptible to bordeaux injury, he found that various organic salts, especially malic acid and potassium, were exuded through healthy uninjured leaves and fruits. However, none of these substances was capable of dissolving any more than insignificant amounts of basic copper. In fact, similar traces of copper were dissolved by pure water containing carbon dioxide, and by water from the surfaces of corked-over organs, such as twigs, which did not exude any such substances. Ruhland was unable to demonstrate the presence of exuded sugar. He therefore concludes that the traces of soluble copper on the leaves are brought into solution by rain water containing carbon dioxide and other substances from the air.

Crandall (1909) states that he was unable to obtain any evidence that the solution of copper occurs through the agency of leaf secretions. No direct experiments are mentioned.

Bedford and Pickering (1910:109 112) planned experiments in which leaves of apple, pear, and damson plums were placed in suspensions of copper carbonate and copper oxide. The leaves were later removed and tests were made for the presence of soluble copper. Solutions in which leaves, apparently uninjured, had been placed, showed the same small quantity (0.00001 per cent to 0.00002 per cent) of soluble copper as did the controls. However, when the leaves were injured or ground up, as much as 0.009 per cent of copper was found in solution. These investigators conclude that there is no evidence that leaf excretions ordinarily are agencies in the solution of copper. The results do not appear to warrant such an unmodified conclusion. Unfortunately the leaves were detached in all cases.

Barker and Gimingham (1911) placed healthy shoots of gooseberry, pear, currant, and apple, while still attached to the trees, in jars containing a small quantity of bordeaux mixture in distilled water, and afterward, on analysis of the water, could detect no soluble copper. However, when no-excess-lime bordeaux was used, very appreciable quantities of soluble copper were detected. The controls showed none. Parallel tests in the laboratory with detached leaves gave the same results. Leaf scorching was correlated with the presence of soluble copper. Later experiments by these investigators (1914 a, 1914 b, 1914 c), on bordeaux injury to apple leaves, showed that, in the case of leaves from summer foliage, scorching by bordeaux resulted only at spots where an injury, naturally or purposely made, allowed contact between the copper compound and the inner thin-walled cells. With leaves from late autumn foliage, the scorching was more general. The injury from no-excess-lime bordeaux was much more pronounced in all cases. When uninjured apple leaves were immersed directly in copper-sulfate solutions, very little general injury resulted. Barker and Gimingham concluded that injured foliage possesses a solvent action not exhibited by uninjured foliage. Presumably this is because the interior cells have readily permeable walls unlike the heavily cuticularized outside walls of the epidermis.

The experimental evidence on the hypothesis of solvent action by suscept excretions is meager. There seems to be no satisfactory proof that uninjured leaves exercise any solvent action. Injured leaves, on the other hand, appear in all cases to exert some solvent action. However, it must be borne in mind that fungicidal toxicity is definitely exhibited in vitro, so that even though the excretions from injured leaves may be contributory to the solution of copper, they cannot be the sole agents.

SPORE EXCRETIONS

Since the present investigations have been primarily directed toward a study of the hypothesis that spore excretions exert the necessary solvent action on the compounds, a fairly comprehensive review of the literature is presented.

Swingle (1896) was apparently the first to suggest the action of the fungus spores themselves. He states (page 21 of reference cited):

> Finally, it is possible that substances secreted or excreted by cells of parasitic fungi may effect the solution of a small amount of copper. The ferments and cements secreted by the

hypha tips of many parasitic fungi, especially when they come into contact with a solid body and in case the solid particle contains copper, might dissolve a considerable amount just about the delicate growing tip.

If, as is quite possible, the copper is quickly absorbed by the fungous cells, the solvent could then take up another portion, which in turn would be absorbed by the fungus, and so on. In this manner poisoning might ensue, even though the copper were only very slightly soluble.

Frank and Krüger (1894) found that spores of different Peronosporaceae and other fungi were killed when immersed for 24 hours in bordeaux mixture several weeks old. However, when similarly treated with the filtrate from the mixture, they were not killed, but germinated. The filtrate showed no soluble copper by chemical tests, and these authors conclude that the presence of the insoluble copper is necessary to bring about the death of the spores.

Rumm (1895) placed uredospores of *Puccinia coronata* in drops of bordeaux mixture. Those spores not in contact with the bordeaux particles, not only were not injured, but germinated. Only when the spores were in contact with the particles or immediately over them, were they killed. As a result of this test, Rumm believes that bordeaux mixture is not fungicidally active at a distance from the particles. However, Doran (1923), as well as workers in the Cornell laboratory, have found that the uredospores of grain rusts are not very sensitive to the toxic action of copper fungicides.

Aderhold (1899) obtained some interesting results while studying the poisonous action of bordeaux mixture. He found that spores of *Fusicladium pirinum* would germinate on the surface of pear leaves sprayed with bordeaux mixture, provided the spores were between the flecks of bordeaux, but that there was no germination directly over the flecks. Also, the spores would germinate in drops of rain water collected from leaves that had been sprayed. Drops of bordeaux mixture were dried down, and strips of filter paper, moistened with rain water and bearing spores of Fusicladium, were placed with one end on the dried bordeaux drops. At a distance of 0 to 4 millimeters from the bordeaux drop, no germination occurred. Progressing farther away from the bordeaux, the germination improved, and at 10 to 13 millimeters distant it was normal. Aderhold believes that the fungicidal action is in evidence only during the formation of the germ tube or during invasion itself.

Clark (1902:41) appears to have followed Swingle's suggestion with an experiment. Spores of various fungi were placed in a mixture of copper hydroxide and water in which no soluble copper could be detected beforehand. The spores were killed in a short time, and traces of copper were found in the suspension, thus "showing that they dissolve more of the $Cu(OH)_2$ than was required to kill them" (page 42 of reference cited). No controls are mentioned. Clark found further that thin-walled spores, such as those of Rhizopus and Oedocephalum, are more quickly killed than thicker-walled ones such as those of Aspergillus and Penicillium. Clark (1902:43) concludes:

> 1. The solution of that part of the $Cu(OH)_2$ of bordeaux mixture which under orchard conditions *is of fungicidal value, is chiefly accomplished by the solvent action of the fungus spores themselves,* for they have power to dissolve sufficient copper to kill themselves.
>
> 2. The amount of copper necessary for the destruction of the spores of parasitic fungi is probably not more than one part of soluble metallic copper to 80,000 parts water (dew).

This work was rather severely criticized by Bedford and Pickering (1910: 108 109), who, in addition, showed that copper hydroxide is slightly soluble (0.00012 per cent soluble copper) in water exposed to the air.

Schander (1904) made tests in which bordeaux mixture was sprayed on various kinds of leaves exposed to the rains and dews, and the filtrates from these were tested for soluble copper. Schander (1904:526) concludes (translation of original): " Upon the grounds of my experiments I do not believe that enough soluble copper compounds form upon the leaf to kill the spores of fungi, but I assume with Clark that, on the whole, fungi dissolve enough copper from the precipitate of bordeaux mixture adhering to the leaf to kill themselves."

Ruhland (1903), studying fungicidal action in bordeaux mixture and other basic coppers, concludes that the rôles of atmospheric agencies and plant excretions are practically negligible, but that (translation of original) " in fact the spore excretes substances which bring the $Cu(OH)_2$ into solution. The copper dissolved by the excretion which has its origin in the metabolic activities preparatory to germination now penetrates the spores"(page 199 of reference cited). The conclusion "that the liberation of the toxic quantity of copper is brought about through the excreted metabolic products of the pre-germination activities of the spore " (page 191 of reference), is based primarily on experiments in which germination tests were made with spores of *Aspergillus niger*, *Botrytis vulgaris*, Clasterosporium, and *Cephalothecium roseum*, in (1) the control, a 0.3-per-cent glucose solution, (2) the filtrate from a 24-hours-old suspension of $Cu(OH)_2$ in a 0.3-per-cent glucose solution, (3) the filtrate from a 24-hours-old suspension of $Cu(OH)_2$ in a 0.3-per-cent glucose solution in rain water, and (4) a 0.3-per-cent glucose solution containing a suspension of $Cu(OH)_2$ and many spores. Though not definitely stated, the water in (1) and (2) presumably was not rain water. After standing thus for 48 hours, the results were, for all four fungi, 100 per cent germination in (1) and (2), and from about 50 to 100 per cent germination in (3). However, Ruhland states that in (3), 100 per cent germination would result if the spores were left for three days. None of the fungi germinated in (4). In the case of (4), soluble copper could be detected in the filtrates by testing with concentrated sulfuric acid and potassium bromide. Apparently the spores had absorbed, or otherwise removed from solution, only a part of the copper that they had dissolved.

Crandall (1909:291) reports, "No evidence has been obtained in support of the suggestions of authors that solution of copper occurs through the agency of germinating spores." However, no direct experiments on which to base this conclusion are mentioned.

Barker and Gimingham undoubtedly have undertaken the most extensive investigations relative to the solvent action of fungus spores. In one series of experiments (1911), spores of *Penicillium glaucum* were placed in a 1-per-cent dextrose solution plus insoluble basic copper sulfate. After a time this showed 0.0011 per cent of soluble copper, or twice as much as the controls. Similarly, Penicillium spores were placed in a dextrose solution, and, after fair growth, were filtered. The filtrate was then added to the basic copper sulfate, and 0.0075 per cent of copper was brought into solution as compared with the control, which showed 0.0004 per cent. The authors, however, point out that the conditions were abnormal in that the solvent action may have come from dead or dying cells, and also that the culture reaction was acid. Hanging-drop cultures of conidia of *Nectria ditissima* and *Sclerotinia fructigena* were made. In general, in the presence of no-excess-lime bordeaux ($10CuO, SO_3$) or of ordinary bordeaux, there was little if any germination; while in the filtrates from such compounds, there was usually good germination and fair growth, though not so good as in the controls. But with uredospores of *Puccinia hieracea*, in the presence of no-excess-lime bordeaux there was some germination and fair growth, while in the filtrates and the controls the percentage germination was high and the growth of germ tubes vigorous. The authors state (pages 86–87 of reference cited):

> The whole of these results tend strongly to support the hypothesis that certain fungi at least are able to act on the insoluble copper compound sufficiently to poison themselves, provided that they are associated closely enough with it: for in all cases copper was practically absent from the filtrate before use, so that it was obviously the presence of the insoluble compound which was necessary to give the mixture fungicidal properties. There appear to be only two explanations to satisfy the results. Either the fungus spore in its dormant condition or in the incipient stages of germination exerts a solvent action on the copper compound: or the minute traces of that substance which can be present in a state of solution act as a cumulative poison.

In another experiment, no-excess-lime bordeaux was placed inside a diffusion tube, and the filtrate on the outside. Nectria spores were sown in each, and germinated well on the outside. A few germinated well on the inside where they were not in contact with the copper particles, which had, however, largely settled. Again, no-excess-lime bordeaux was dried on glass cover slips and suspensions of Nectria spores in distilled water were added so that they overlapped the bordeaux films. The water was allowed to evaporate and the cover slips were then placed in a moist atmosphere. The protoplasm of the spores on the film of bordeaux became badly shrunken, while the spores beyond the zone of bordeaux were apparently normal and many had germinated. From these results Barker and Gimingham conclude (pages 88 89 of reference cited):

> It seems reasonable to postulate, therefore, a solvent action on the part of the fungus which is not powerful enough normally to produce serious toxic effect except when direct contact occurs. At the same time it is evident that such action is purely local and cannot result in the bringing into solution of sufficient copper to exercise a general fungicidal action over the whole surface of a leaf.

Pickering (1912) has criticized in some detail Barker and Gimingham's (1911) conclusions, and points out that the majority of their experiments were with the no-excess-lime bordeaux ($10CuO, SO_3$), which is not entirely insoluble. These criticisms have been sharply refuted by Barker and Gimingham (1914 b).

The later work of Barker and Gimingham (1913, 1914 b) is concerned chiefly with seedling root-hairs, the action of which they consider comparable to that of germinating spores. The seedlings were placed in various copper-compound suspensions, chiefly no-excess-lime bordeaux. Ordinary bordeaux and copper carbonate also were used. Barker and Gimingham found that only when the root hairs were in contact with the particles of the copper compound did injury and death result. The root hairs not in contact escaped injury and grew. The injurious action was entirely local, and, as the authors state (1913:61), therefore "cannot be explained as a result of the production of soluble copper by atmospheric agencies." The authors emphasize the action of the cell wall, and finally conclude, in part (page 62 of reference cited):

> (1) Living cells with readily permeable walls of the unchanged cellulose type or its equivalent are able to produce and absorb soluble copper from insoluble compounds such as the basic sulphates.
>
> (2) The area over which a single cell can exert the solvent action is limited by the size of the cell or, perhaps, more accurately, by the quantity of the solvent diffusing from it. Groups of cells acting in conjunction may cause appreciable action over a wider area than an isolated cell singly.
>
> (3) The fate of the organism depends upon the relation between the amount of soluble copper produced and absorbed and the rate of growth of the organism.

Butler (1914:139), in view of his experiment with distilled and tap water which has already been discussed under atmospheric agencies, assumes that the sporangia of Phytophthora or of Plasmopara could not have an excretory solvent action, "for, so far as at present known, organic solvents of the copper precipitate of bordeaux mixtures are more active in alkaline than in neutral mixtures, and their action is not materially interfered with when tap-water is used in lieu of distilled water."

Lutman (1916) made some interesting studies, but did not definitely commit himself to any of the three hypotheses. He employed spore tests using spores of *Phytophthora omnivorum*, *Sclerotinia cinerea*, and *Ustilago* *levis.* Bordeaux mixture dried on glass slides was exposed to the rain and dew to test the effect of exposure. Spores were then placed on the slides, and, though toxicity was evident, it was but very little more pronounced than in the unexposed controls. The supernatant water from exposed bordeaux mixture was found non-toxic. The toxicity of lime-water was investigated, and the alkalinity was found to be sufficient to inhibit the germination of the spores. The effect of distance from a bordeaux film also was studied. Spores of Phytophthora and Sclerotinia were sown on glass slides at certain distances from films of ordinary bordeaux or no-excess-lime bordeaux. It was found that the spores nearest the films, that is, within 1 millimeter, did not germinate. But as the distance from the bordeaux film was increased, so the percentage of germination and the growth of germ tubes increased. Copper wire also was found to inhibit the germination of these spores if they were present in its immediate neighborhood. Lutman is inclined to the view that the copper salts formed in bordeaux mixture are, when moistened, very slowly soluble (pages 34-35 of reference cited). He planned no experiment, however, to demonstrate this. His final conclusions are:

1. The fungus spores are killed by contact, either:
   (a) with the bordeaux film, giving off free copper; or,
   (b) with the alkaline solution derived from some of the lime particles.
2. The spores are inhibited from germination if they are in the region either of the bordeaux film or of the lime particles.

Cerasoli (1921) placed grape leaves which were, respectively, healthy, slightly affected with mildew, and withered with mildew — the last-named two being well covered with fungous growth — in distilled water, care being taken to cover the leaf blades only. After some days of exposure to the air, it was found that only the water containing healthy leaves gave a neutral reaction, the water in the other cases being more or less acid. The acidity was most pronounced with the leaves having the greatest amount of fungous growth. From this Cerasoli concludes that the solution of the copper salts is due to very weak acids or acid salts secreted by the parasite during its entry into the tissues, and that this acidity would easily react on the colloidal copper hydrates which he claims are formed in bordeaux mixture, thus giving rise to copper ions to which the fungicidal action supposedly is due.

Schmidt's work (1924) has already been discussed under atmospheric agencies. He was unable to demonstrate the secretion of solvent substances from spores of Botrytis, Aspergillus, Monilia, Fusicladium, and Trichothecium, that would dissolve the copper in copper hydroxide or bordeaux mixture.

The evidence to be derived from the literature relative to the hypothesis of fungous excretions is very conflicting. Some investigators apparently have clearly demonstrated this solvent action. Others have been unable to find any such evidence. All the positive evidence seems unanimous in showing that the sphere of action of the spores is decidedly limited. As to the nature of the solvent excretions, if any, there appears to be no experimental information.

OTHER HYPOTHESES

A hypothesis that has received but scant mention in literature is one which might be called *cumulative action*. This applies to very difficultly soluble or even so-called insoluble fungicides. It is based on the assumption that absolute insolubility is impossible. Theoretically, all compounds in the presence of water may be expected to enter into solution to a certain extent. The actual amount in solution may be the merest trace, but it will exist in equilibrum with the insoluble part. If the solute be removed, it is assumed that more will enter into solution to maintain the equilibrum. In this manner an appreciable amount of the compound may eventually come into solution, provided always, however, that the solute is constantly removed from solution.

Swingle (1896) perhaps has this hypothesis in mind when he suggests that the protective action of bordeaux mixture is due to copper in solution which so weakens the germ tube of the fungus as to render penetration impossible. Swingle cites much of Nägeli's work (1893) on oligodynamic action, or poisoning by exceedingly small quantities of metallic substances in solution. Lutman (1916) is inclined to favorably consider Swingle's suggestion. On the other hand, Butler (1914) was unable to demonstrate any evidence of oligodynamic action.

Bell and Taber (1907) hold that minute traces of copper are in solution in bordeaux mixture, and that the concentration of this soluble copper is constant.

Barker and Gimingham (1911:86-87) state that the minute traces of copper in solution in bordeaux mixture may act as a cumulative poison. However, in a later paper (1913:59), they state that the minute traces of copper " which are present in solution" did not "act as a cumulative poison."

Pickering (1912), in his studies on the action of iron rods on the solubility of 10CuO, SO_3, is inclined to this hypothesis. He was, as has been noted, able to render small amounts of copper soluble, and in discussing the low degree of solubility of 10CuO, SO_3 he makes the significant statement (page 280 of reference cited):

> The greater part of the copper in it may pass into solution and be utilised within a comparatively short time, provided there is any body present, such as iron, to react with it and remove it from the solution. This will occur equally if it be removed by absorption into a fungus spore or the leaf of a plant, and the absorption in that case should be at a much greater rate than in these experiments, because the reacting surface exposed to a given amount of copper would be far greater. . . . . Thus we may get a very energetic absorption or

fungicidal action, with a substance showing a minimum of solubility, though the possibility of this action depends entirely on the substance passing into solution.

Barker and Gimingham (1914 b:223), as a result of their work on the localized action of solvent excretions from fungus spores, say: "Pickering's explanation that death is due to absorption of minute traces of copper in solution in the continuous film of liquid covering the preparation absolutely fails to explain such cases."

There are a number of other hypotheses related to fungicidal action. They are not concerned directly with the solution of the copper, however, but primarily with the toxicity of copper. Since a study of the principles of toxicity is not within the scope of this paper, they are not discussed here.

Some workers, notably the Villedieus (1920a, 1920b, 1924), and G. Villedieu (1921, 1924), have maintained that bordeaux mixture owes its protective action almost entirely to its basicity. There is little experimental evidence in support of this hypothesis.

Hooker (1924), studying the fungicidal properties of colloidal copper hydrate, suggests that the action of the copper is a catalytic one promoting oxidation. This is similar to the view of Dubois (1923), that copper and its salts appear to destroy fungi by an action comparable to that of oxidases and peroxidases and not by direct toxic action. No experimental proof was offered in either case.

The view, especially held by a number of German investigators, that the copper is absorbed by the leaf and is thus present to protect the suscept from invasion, is not dealt with here. In any case the copper must presumably be in a soluble form in order to enter the leaf. We are thus still faced with the question of the solution of the copper.

EXPERIMENTAL WORK

PROCEDURE

In the studies conducted at this laboratory, various methods of approach have been used in an effort to detect the exertion of solvent action by the spores of fungi. The methods are similar, however, in that spore tests have been universally employed; that is, spores have been used directly to detect the presence of soluble toxic materials. We are here concerned with such minute quantities of the toxic substances that the usual chemical tests have been found inadequate. However, certain types of spores will give a very definite reaction to the presence of deleterious substances in extraordinarily small amounts. This is especially so in the case of copper. Furthermore, in nature it is the spore itself which in the final analysis must be the indicator of toxicity.

The general method and technic of testing by spores have been discussed in detail in the second paper of this series. The procedure there described has been followed throughout, with the simple modification that the aqueous suspension of spores was added to the solution or suspension to be tested. The resulting spore suspension was then pipetted as drops on the slides, and the germination tests were thus made. In order to prevent undue dilution, a concentrated spore suspension usually was added to the solution or suspension before the latter was placed on the slides. Commonly one volume of spore suspension was added to four volumes of solution or suspension; thus the latter was diluted to four-fifths of its original strength.

In all cases, for a specific test the percentage germination is based on the counts from at least sixteen drops. Usually, however, this number has been doubled, and not uncommonly it is the average of three or four tests. The results are recorded as explained on page 21; that is, the percentage and the type (A, B, C, or D) of germination are recorded. The A type of germination is the most vigorous, having germ tubes of approximately 200μ in length or longer; in B type, the germ tubes are 100 to 200μ long; in C type, they are 25 to 100μ long; and in D type, they include those just produced and those up to 25μ in length.

It might be well to state here that the writer agrees with Clark (1902:29), that it is more desirable to consider the point of inhibition of germination rather than the death point, for, "if the germination of the spores be *inhibited* by a concentration of a copper salt, it was merely a question of time when they would be killed."

After experimenting with various fungi, conidia of the fungus *Sclerotinia americana* were finally selected as being the most suitable for these investigations. *Sclerotinia americana* (Wormald) Norton and Ezekiel is the common brown-rot fungus of stone fruits in North America (Ezekiel 1924, Wormald 1928a, 1928b). In culture it produces an abundance of fairly large conidia. These spores germinate readily in water and with a high percentage of germination. Standardized cultures (page 13) giving from about 97 to 98 per cent germination were used in all tests. The spores were from cultures five to twelve days old; distilled water was used for spore suspensions; concentrations of spore suspensions showed from fifteen to forty spores per microscopic field of 1575μ diameter; incubation was at a temperature of 21° to 26° C.; and spore-germination counts were taken from 24 to 36 hours after planting. In certain cases, tests with other fungus spores supplemented those made with *S. americana*.

THE TOXICITY OF COPPER

On the selection of *Sclerotinia americana* conidia for these investigations, it seemed desirable to obtain more nearly accurate information relative to the toxicity of copper toward these spores. For this purpose, dilute solutions of copper sulfate in distilled water were made up. At infinite dilution, the ionization of this salt is presumably complete (Randall, 1916). Hence, at the dilutions used in these tests — that is, 0.1 to 10 milligrams of copper per liter of copper-sulfate solution — the copper sulfate is from 96 to practically 100 per cent ionized, or nearly all in the ionized state.

In studying the toxicity of copper-sulfate solutions toward spores, at least four factors must be considered. These are: the age of the spores; the number of spores; the concentration of copper in solution; and the volume of solution.

In table 1 is recorded the germination of spores of different ages in varying dilutions of copper-sulfate solutions. These figures are based on the results from a number of tests. The tests are in close agreement. The concentration of the spore suspensions was from approximately 30 to 32 spores per low-power field of the microscope. In all future references to concentration of the spore suspension in this paper, the number of spores is designated for a low-power field. The low-power field of the microscope employed is 1575μ in diameter. The area of the field is thus approximately 1/30 of that of the entire drop, since the drops are from 8 to 10 millimeters in diameter. Also, since the drops are 1/20 cubic centimeter in volume, the volume of solution in a low-power field would be approximately 1/600 cubic centimeter. The temperature was from 21° to 25° C.

TABLE 1. PERCENTAGE GERMINATION OF SCLEROTINIA AMERICANA CONIDIA IN DILUTE COPPER-SULFATE SOLUTIONS: RELATIONS OF COPPER-SULFATE CONCENTRATION AND SPORE AGE

(Spore-suspension concentration, 30 to 32)

Age of spores, in days	Milligrams of copper per liter of solution					
	0	0.1	0.2	1.0	2.0	10.0
	Per cent and type of germination					
5	98.3 A	98.8 A	85.8 B	0.7 D	Trace	0
6	98.7 A	98.8 A	88.3 B	0.3 D	Trace	0
7	98.4 A	98.6 A	90.0 B+	0.5 D	Trace	0
8	97.8 A	98.1 A	88.2 B	0.8 D	Trace	0
10	96.6 A	96.8 A	76.9 B	0.8 D	Trace	0
12	95.3 A	92.7 A-	64.0 B-	0.3 D	0	0
17	88.5 B	79.0 B	48.3 C	0.3 D	0	0

The results, which are shown graphically in figure 1, immediately demonstrate the extreme sensitivity of *Sclerotinia americana* conidia to copper. As may be expected, the spores become less viable and more copper-sensitive with increasing age. For the younger spores, 5 to 10 days old, 0.1 milligram of copper per liter of solution is slightly stimulating. Increasing the concentration above this results in a rapid decrease in the percentage germination. Spores of the ages commonly used, and

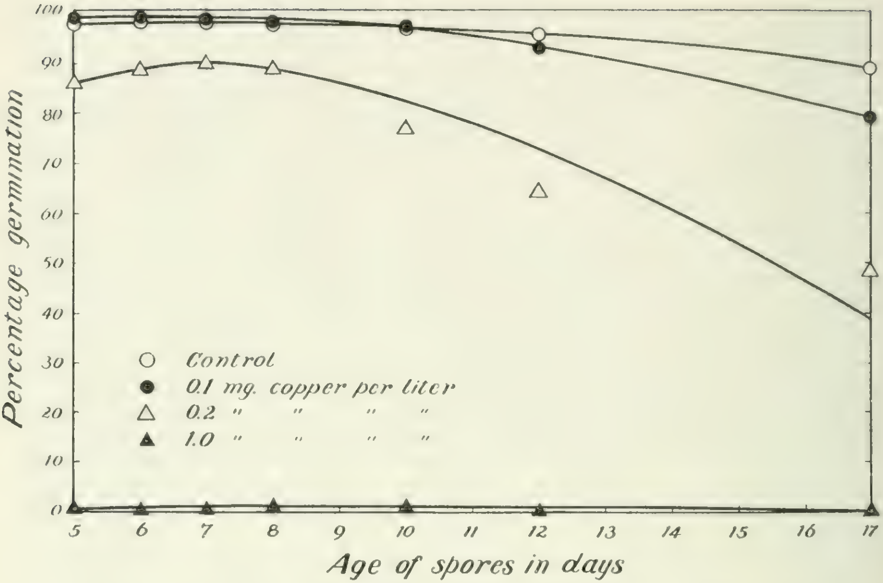

Figure 1. germination of conidia of sclerotinia americana in dilute copper-sulfate solutions: relations of copper concentration and age of spores

sown at a suspension concentration of 30 to 32, are therefore definitely sensitive to as small a quantity of copper as 0.2 milligram per liter of solution. The potassium-ferrocyanide test commonly used for copper will show fairly plainly at 20 milligrams of copper per liter, and with uncertainty at 10 milligrams per liter. The Sclerotinia conidia are therefore, under these conditions, one hundred times more sensitive to copper than the potassium-ferrocyanide test.

The second step was to determine the effect of the number of spores on germination in the dilute copper solutions. The volume of solution and the spore age were held constant. Spore suspensions were obtained, of such concentrations that when they were added to the copper-sulfate solutions the resulting concentrations were as nearly as possible 4, 8, 16, and 32 spores per field. The variation from these numbers was small, being not more than 2 or 3 for the dilute concentrations, and 6 or 7 for the most concentrated. In all, five tests in duplicate were made. The ages of the spores were 7 and 8 days. Copper-sulfate solutions of from 0.1 to 10 milligrams of copper per liter of solution were employed. The results of these tests are recorded in table 2.

TABLE 2. PERCENTAGE GERMINATION OF SCLEROTINIA AMERICANA CONIDIA IN DILUTE COPPER-SULFATE SOLUTIONS: RELATIONS OF COPPER-SULFATE CONCENTRATION AND NUMBER OF SPORES

(Spore ages, 7 and 8 days)

Milligrams of copper per liter of solution	Spore-suspension concentration			
	4	8	16	32
	Per cent and type of germination			
0.0	98.1 A	98.3 A	98.3 A	98.2 A
0.1	3.2 C–	58.6 B–	91.9 A–	98.8 A
0.2	Trace	4.1 C–	54.2 B–	94.5 A–
0.5	0	Trace	0.5 D	36.5 B-
1.0	0	0	Trace	0.6 D
2.0	0	0	0	Trace
10.0	0	0	0	0

These results are shown graphically in figure 2, where the percentage germination is plotted against the concentration of copper. The curves for the four concentrations of spore suspension are drawn.

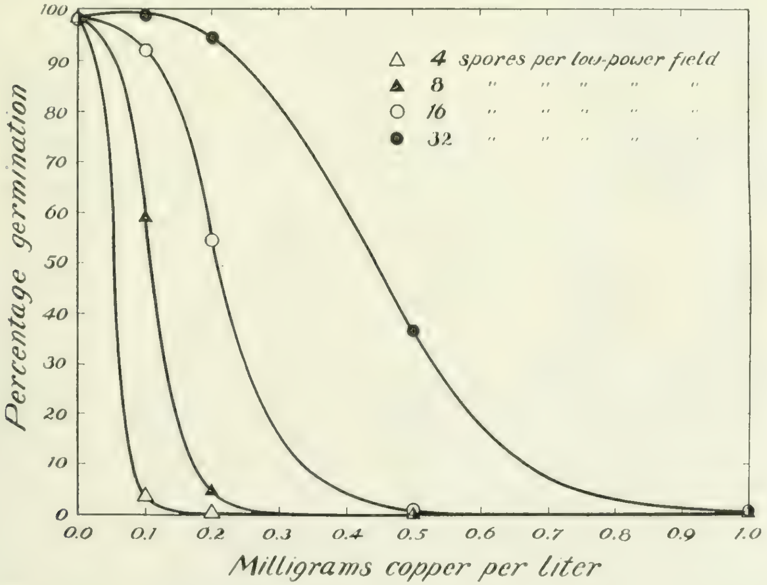

FIGURE 2. GERMINATION OF CONIDIA OF SCLEROTINIA AMERICANA IN DILUTE COPPER-SULFATE SOLUTIONS: RELATIONS OF COPPER CONCENTRATION AND NUMBER OF SPORES

In order that the relations between the number of spores and the total amount of copper may be the more clearly shown, figure 3 has been constructed from figure 2. In figure 3, milligrams of copper per liter of solution are plotted against number of spores, and four germination curves — 0, 5, 50, and 90 per cent — as derived from figure 2 are drawn.

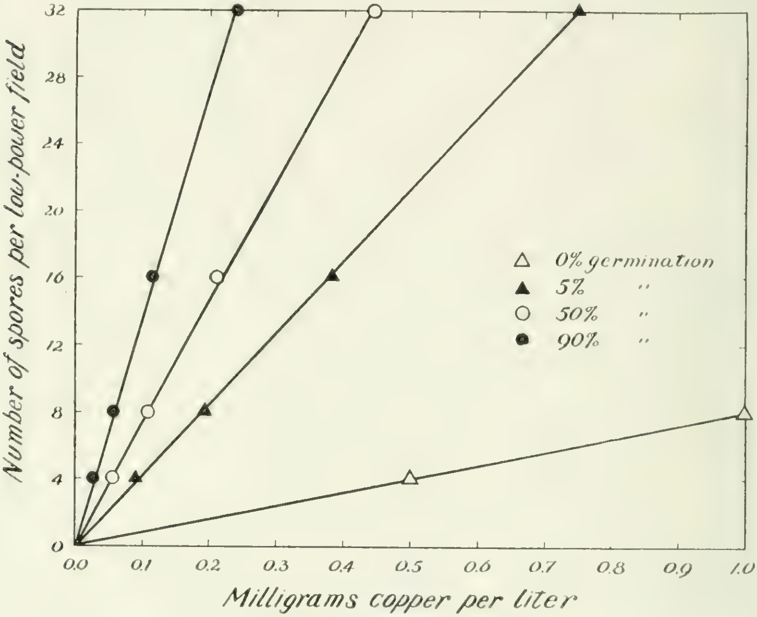

Figure 3. toxicity of dilute copper-sulfate solutions to the conidia of sclerotinia americana

Thus these are shown to be straight-line relations; that is, for a given percentage of germination the number of spores is directly proportional to the concentration of copper. This, of course, assumes that the volume of copper-sulfate solution is constant and small. In other words, there is a direct proportion between the number of spores and the total amount of copper for a given percentage germination.

In the study of the volume of solution, some difficulty was experienced. The spores will germinate well only in small volumes of water. In large volumes the oxygen relations appear unsatisfactory. A "drip system" was finally devised for a study of the larger volumes. Essentially this consists in the dripping of a large volume of solution over the spores, which are held in very shallow petri dishes. The control spores give fairly satisfactory germination here. For the medium volumes, that is, from 1 to 25 cubic centimeters, petri dishes were employed. The smallest volumes were placed directly on slides and a drop of spore suspension was added. With the large volumes, the drops of spore suspension were first placed on the bottom of empty petri dishes and allowed to stand for about thirty minutes before the solution was added. The spores had then settled to the surface of the dish, to which they adhered, and were not readily dislodged. They were thus easily found for the purpose of making germination counts.

In this manner two sets of tests were performed. A copper-sulfate solution of a concentration of 0.1 milligram of copper per liter, was employed throughout. The volumes of solution are expressed as drops, in order that the ratio of drops of solution to one drop of spore suspension may be readily seen. The concentration of spore suspension prior to the dilution was constant and was approximately 16. The age of the spores was 7 days. The percentage of germination observed in these tests is recorded in the third column of table 3.

TABLE 3. PERCENTAGE GERMINATION OF SCLEROTINIA AMERICANA CONIDIA IN DILUTE COPPER-SULFATE SOLUTIONS: FACTOR OF SOLUTION VOLUME

(Concentration of solution, 0.1 milligram of copper per liter; spore-suspension concentration, 16; spore age, 7 days)

Volume of solution: ratio of drops of spore suspension to drops of copper-sulfate solution	Total copper concentrated into one drop and expressed as milligrams per liter	Per cent of germination observed	Per cent of germination theoretically to be expected from figure 2
1:0	0.0	98.2 A	98.3
1:1	0.05	98.6 A	97.0
1:2	0.13	97.8 A	86.0
1:5	0.42	94.5 A–	3.0
1:10	0.91	81.8 B	1.0
1:20 (1 cc.)	1.9	3.9 D	0
1:50 (2.5 cc.)	4.9	Trace	0
1:100 (5 cc.)	9.9	0	0
1:200 (10 cc.)	19.9	0	0
1:500 (25 cc.)	49.9	0	0
1:1000 (50 cc.)	99.9	0	0
1:2000 (100 cc.)	199.9	0	0
1:5000 (250 cc.)	499.9	0	0
1:10000 (500 cc.)	999.9	0	0

A study of these figures shows the importance of the volume factor. A small volume of very dilute copper-sulfate solution may be entirely nontoxic toward a given number of spores, but a large volume of this same concentration is decidedly toxic to the spores. This confirms the observations noted before, that toxicity is governed by the number of spores and the total amount of copper present, and not by the relative concentration. Similar conclusions have been drawn by Burrill (1907:568–569), Briggs (1923), and others.

In order that an adequate comparison of the volume relations may be made, the total copper present in these solutions has been calculated on the basis of milligrams of copper per liter of solution. That is, the total copper present is assumed to be concentrated into a volume of one drop. Having the concentration of copper expressed thus, by a reference to figure 2 the germination theoretically to be expected for 16 spores may be found. These germinations are given in the fourth column of table 3.

A comparison of the observed and the theoretical germination shows a great difference. Over the narrow range of volumes at which germination occurred, it will be seen that the observed germination was approximately equivalent to the theoretical for a volume only about one-fourth as great. The same total amount of copper present in a very large volume of solution is therefore less toxic than that present in a small volume.

This will appear contradictory to the previous statement, namely, that toxicity is dependent on the total copper present regardless of volume or concentration. Thus it would seem that toxicity is not entirely independent of volume, the total copper content being the same. This difference may be explained as being due to the rate of diffusion. In the case of a large volume of a dilute solution, the particles, or ions, of copper are presumably rarer in a given space than they would be in a small volume containing the same number of copper ions. Thus, in the former case, in the proximity of the spore there would be present fewer ions than in the latter case. So the final toxicity would, in a sense, be the resultant of the rate of diffusion of the copper ions and the rate of germination of the spores. The toxicity of a dilute copper-sulfate solution toward a given number of spores is therefore determined, not only by the total amount of copper present, but also to a certain extent by the rate of diffusion of the copper ions.

The results of these preliminary studies have shown that the toxicity of dilute copper-sulfate solutions to the conidia of *Sclerotinia americana* is dependent, not only on the concentration of the solution, but also on the age of the spores, the number of the spores, the total amount of copper present, and the volume of the solution. With increasing age the spores become more copper-sensitive. The toxicity, or inhibition from germination of a given number of spores, is directly related to the total amount of copper present in a given volume of solution. With the total copper content and other factors held constant, the solutions become less toxic with increasing volumes, the limiting factor being presumably the rate of diffusion of the copper.

Spores from 5 to 10 days old, present to the extent of less than 1000 to a drop of solution, are markedly sensitive to 0.2 milligram of copper per liter of solution. This is one hundred times more sensitive than the potassium-ferrocyanide test.

THE TOXICITY OF CALCIUM COMPOUNDS

Since a number of the common copper protectants contain calcium in various forms, a few preliminary tests were made to determine the relative toxicity of certain of these calcium compounds. The calcium compounds present in excess-lime bordeaux mixtures and copper-lime dust are calcium hydroxide, calcium sulfate, and calcium carbonate. Calcium hydroxide and calcium sulfate are both moderately soluble in water at 25° C., the former to the extent of 1.541 grams per liter expressed as $Ca(OH)_2$, and the latter 2.755 grams per liter expressed as $CaSO_4 \cdot 2H_2O$ (Cameron and Bell, 1906). Calcium carbonate is soluble in distilled water only to the extent of 19.6×10^{-5} gram mols per liter at 25° C. (Askew, 1924).

Four experiments were performed with calcium hydroxide, the first two in triplicate and the third and the fourth in duplicate. A saturated solution of $Ca(OH)_2$ was obtained by slaking a high grade of commercial stone lime with distilled water, making milk of lime, and filtering. From this filtrate various dilutions were made. This source of $Ca(OH)_2$ was employed in the first three experiments; for experiment 4, Baker's Chemically Pure $Ca(OH)_2$ was used. The toxicity of all was determined in the usual manner. The results are presented in table 4.

TABLE 4. PERCENTAGE GERMINATION OF SCLEROTINIA AMERICANA CONIDIA IN CALCIUM-HYDROXIDE SOLUTIONS

Concentration of $Ca(OH)_2$ solution, expressed as per cent of saturation	Commercial $Ca(OH)_2$			Baker's Chemically Pure $Ca(OH)_2$
	Experiment 1	Experiment 2	Experiment 3	Experiment 4
	Per cent and type of germination			
100	0	0	0	0
50	Trace	Trace	0	Trace
25	17.3 A–	31.0 A–	4.3 A–	1.2 A–
12	96.3 A	92.1 A	93.4 A	26.5 A
6	98.4 A	98.6 A	98.6 A	97.0 A
Control	98.3 A	98.6 A	98.4 A	98.0 A
Age of spores	5 days	7 days	5 days	5 days

Similar tests were made with calcium-sulfate solutions. The calcium sulfate was obtained from three sources — Bausch and Lomb C. P., Merck precipitated, and Baker technical. The results are shown in table 5.

TABLE 5. Percentage Germination of Sclerotinia americana Conidia in Calcium-Sulfate Solutions
(Spores 5 days old)

Source of $CaSO_4$	Concentration of $CaSO_4$ solution, expressed as per cent of saturation	Experiment 1	Experiment 2
		Per cent and type of germination	
Bausch and Lomb C. P.	100	Trace	Trace
	50	60.8 C	19.9 C−
	25	94.6 A	96.7 A
	12	98.6 A	98.8 A
	6	98.7 A	98.5 A
Merck precipitated	100	11.2 C	2.1 D
	50	97.0 A	96.2 A
	25	98.8 A	98.4 A
	12	98.4 A	98.3 A
	6	98.6 A	98.5 A
Baker technical	100	9.8 C	3.2 D+
	50	96.9 A	70.0 B+
	25	98.4 A	98.6 A
	12	98.6 A	98.6 A
	6	98.5 A	98.3 A
Control	...	98.4 A	98.4 A

The toxicity of calcium carbonate was tested, using the material as a dust. Three tests were made, the slides being dusted in the usual manner. On the addition of the aqueous spore suspension, a saturated solution of the calcium carbonate would presumably result and be present together with the very great excess of solid dust particles. The results are shown in table 6.

TABLE 6. Percentage Germination of Sclerotinia americana Conidia on Slides Dusted with C. P. Calcium Carbonate

	Experiment 1	Experiment 2	Experiment 3
	Per cent and type of germination		
Calcium carbonate	98.3 A	98.3 A	98.5 A
Control	98.4 A	98.4 A	98.5 A
Age of spores	9 days	9 days	9 days

These tests show that a clear saturated solution of calcium hydroxide in water is decidedly toxic toward conidia of *Sclerotinia americana*. Normal germination is obtained only when the solution has been diluted to 6 per cent of saturation.

Solutions of calcium sulfate also are toxic. They are, however, approximately only one-sixth as toxic as similar concentrations of calcium-hydroxide solution. It is perhaps possible that the greater toxicity of the calcium-hydroxide solution is due to its alkalinity. No determination has been made of the possible toxicity of the sulfate radical or ion, either in these tests or in those with the copper sulfate.

Calcium-carbonate dust displays no toxic action toward the spores of *Sclerotinia americana.*

THE LIBERATION OF THE COPPER FROM THE PROTECTANT

In studying the manner in which the copper is rendered soluble, various types of experiments have been employed. These experiments have, in turn, been directed toward proving or disproving certain of the more important current hypotheses. In the presentation of each study, the hypothesis is first stated, and this is followed by a statement of the experiments and experimental data, and finally by a discussion of the results.

Special emphasis has been laid on the hypothesis of spore excretions. The first two of these studies have dealt with the action of atmospheric agencies. The next three relate to the rôle of spore excretions, the third of these being concerned especially with the sphere of activity of the spore. Finally, brief studies have been made of the cumulative-action hypothesis.

Atmospheric agencies

The solubility of copper protectants in distilled water

Hypothesis. Copper protectants in distilled water may be brought into solution when normally exposed to the action of the air. A copper protectant is applied to a glass surface, and in one case distilled water is allowed to remain standing on it for varying lengths of time. After exposure thus to the air, spore-germination tests are made in the filtrate. In the second case, a suspension of spores is placed directly on the fungicide and the germination is recorded. If atmospheric factors alone are operative in the solution of the copper, then in the first case there should be little or no germination, and the toxicity of the filtrate should increase with the time of standing; while in the second case, because of the short exposure to the air the germination should be good. However, if an action of the spores is the main factor, then in the first case none of the filtrates, regardless of the time of standing, should be toxic; but in the second case, because of the presence of the copper compound there should be little or no germination.

Experiments. The copper protectants employed in these experiments were bordeaux mixture (4–4–50), Pickering or no-excess-lime bordeaux (2.5–2–50), copper-lime dust, C. P. copper carbonate, and copper hydroxide. The freshly made bordeaux mixtures were tested for free copper by the use of potassium ferrocyanide, and none was found. *Sclerotinia americana* conidia were used in the germination tests.

The fungicide to be tested was sprayed or dusted into open petri dishes. Liquid fungicides were allowed to dry thoroughly. Distilled water was then added to each, about 10 cubic centimeters to a dish, and the whole was allowed to stand exposed to the air for 24 hours. The suspensions were then filtered through filter paper and the filtrates tested for free copper with potassium ferrocyanide. None was found in any test.

Coincidentally, similar but freshly made protectants were applied to glass slides. No free copper was detected here with potassium ferrocyanide. After the liquid fungicide had thoroughly dried, spore-germination tests were performed, (a) in the filtrates and (b) directly on the sprayed or dusted slides.

The average of three tests with bordeaux mixture 4–4–50, no-excess-lime bordeaux 2.5–2–50, and copper-lime dust, are given in table 7.

TABLE 7. PERCENTAGE GERMINATION OF SCLEROTINIA AMERICANA CONIDIA

Treatment	Bordeaux 4–4–50	Bordeaux 2.5–2–50	Copper-lime
	Per cent and type of germination		
Filtrates	94.7 A	98.2 A	93.5 A
Treated slides	0	0	0
Control	98.5 A	99.2 A	98.5 A

Tests were likewise made with copper carbonate and copper hydroxide. The copper carbonate was obtained from four sources and all was C. P. material. The fourth sample was carefully and thoroughly washed and dried before testing. The copper hydroxide, also C. P., was obtained from two sources. Two separate tests, each in duplicate, were made on all of these materials. The spores were from 5 to 7 days old. The results are shown in table 8.

TABLE 8. PERCENTAGE GERMINATION OF SCLEROTINIA AMERICANA CONIDIA

Treatment	C. P. copper carbonate				C. P. copper hydroxide	
	Samples				Samples	
	1	2	3	4	1	2
	Per cent and type of germination					
Filtrates	0	0	0	0	0	0
Treated slides	0	0	0	0	0	0
Control	98.6 A	98.6 A	98.4 A	98.4 A	98.2 A	98.6 A

Discussion. The results of the first series of experiments show clearly that practically no copper goes into solution in the distilled water which stands on the bordeaux mixtures and the copper-lime dust. It therefore seems improbable that atmospheric factors act, at least over a period of 24 hours, through the medium of distilled water to promote the liberation of copper. The possibilities of "meteoric waters" and longer periods of standing have not, of course, been precluded here. However, it is very evident that in those cases in which the fungus spores were placed directly on the insoluble protectant, the latter was decidedly toxic; that is, in some manner the mere presence of the insoluble protectant is sufficient to inhibit the germination of the spores. This may be explained by assuming that the spores excrete a solvent capable of dissolving some of the copper.

The experiments with C. P. copper carbonate and C. P. copper hydroxide resulted contrarily to expectations. These copper compounds are ordinarily considered insoluble in water (Holland and Gilligan, 1928). Apparently this is not strictly true, for it is very evident that the filtrates contain substances in solution which are toxic to the spores. Presumably this toxic material is soluble copper. The filtrates from the copper carbonate gave no copper test when treated with potassium ferrocyanide; the copper-hydroxide filtrates, however, gave a fairly clear test. Thus the amount of copper present in the former is probably less than 10 milligrams per liter, while in the latter it is probably more nearly 20 milligrams per liter. This unsuspected fact of soluble toxic substances precludes the possible use of these copper compounds in subsequent studies of this kind.

The solubility of copper protectants in distilled and rain water, under forced aeration

Hypothesis. It is possible that in nature the copper protectants are more exposed to the action of carbon dioxide and other atmospheric agencies, than they are with plants merely standing in the laboratory. Also, rain waters perhaps possess a greater solvent action than does distilled water.

Experiments. A series of experiments were accordingly performed, in which a prolonged and enforced action of the air and of rain water was tested. Bordeaux mixture (4-4-50) and copper-lime dust were used. The test fungi were conidia of *Sclerotinia americana* and of *Macrosporium sarcinaeforme.* The latter fungus was used only in one test. It was found, however, to be approximately as copper-sensitive as *Sclerotinia americana.*

The fungicides were placed in specimen jars of 175 cubic centimeters capacity. The bordeaux mixture was sprayed on the inner side of the jars, and also on both sides of glass slides. Four of these slides were placed in each jar, this serving to increase the surface exposed. After thorough drying, about 50 cubic centimeters of distilled water was added to each jar. The jars were then connected to a water suction pump in such a manner that fresh air was continually and vigorously drawn through the water, bathing the fungicide-coated surfaces. The water level was kept constant by daily additions of fresh distilled water. A second set was maintained, in which the fungicides were merely placed in open petri dishes and water was added as in the previous method.

In the first and second tests the air was allowed to bubble through for 9 days. In the third test this period was 8 days. At the end of these periods all the suspensions — those in the jars through which air had been passed, and those standing in the open petri dishes — were filtered through ordinary laboratory filter paper. Chemical tests with potassium ferrocyanide and potassium mercuric thiocyanate at this point failed to demonstrate the presence of soluble copper in any of the filtrates. At the outset of the experiments, similar tests of the filtrates from the fungicides had failed to show any soluble copper.

Germination tests were made with the filtrates. Although the filtrates failed to react to the chemical tests, it was thought that sufficient copper might be present to warrant a dilution. Thus, to attempt a differentiation of the filtrates, they were diluted with distilled water to one-half, one-third, and one-quarter strengths. These concentrations — full, one-half, one-third, and one-quarter strengths — are respectively designated as filtrates 1, 2, 3, and 4. The results of the germination tests are shown in table 9.

TABLE 9. Percentage Germination of Spores in Filtrates from Distilled Water on Copper Protectants Exposed to Various Treatments

Experiment no.		1		2		3	
Duration		9 days		9 days		8 days	
Fungus spores		*Macrosporium sarcinaeforme* 15 days old		*Sclerotinia americana* 4 days old		*Sclerotinia americana* 4 days old	
Treatment	Dilution	Air passed through	Standing in open dish	Air passed through	Standing in open dish	Air passed through	Standing in open dish
		Per cent and type of germination					
Bordeaux (4-4-50) filtrates	1	96.3 A	98.6 A	87.0 C	94.1 C	27.6 D	20.0 D
	2	96.6 A	96.3 A	99.1 A	98.6 A	69.3 C	63.0 C
	4	98.8 A	....	98.3 A	....	....	....
Sprayed slides from jar after aeration		0	....	0	....	0	....
Copper-lime filtrates	1	100.0 A	98.6 A	73.2 C	96.5 B	89.4 B	29.0 C
	2	98.8 A	100.0 A	96.5 B	97.9 B+	86.2 B	91.5 A
	4	98.4 A	....	96.3 B+	....	....	....
Controls		99.0 A		98.5 A		98.7 B+	

In a further series (4 and 5), the action of rain water was compared with that of distilled water, the air being bubbled through the jars for 6 and 5 days, respectively. For this purpose fresh rain water was collected. In these tests bordeaux mixture (4–4–50) was used. One bordeaux preparation was made as usual with distilled water, while in a second preparation rain water was used instead. The set-up was similar to that of the former series. There were four combinations — distilled-water bordeaux with distilled water, rain-water bordeaux with distilled water, distilled-water bordeaux with rain water, and rain-water bordeaux with rain water. A second set in open petri dishes was maintained as before. Chemical tests before and after showed no signs of free copper. The results of the germination tests with the filtrates, using conidia of *Sclerotinia americana*, are recorded in table 10.

TABLE 10. PERCENTAGE GERMINATION OF SCLEROTINIA AMERICANA CONIDIA IN FILTRATES FROM RAIN WATER AND FROM DISTILLED WATER, ON BORDEAUX MIXTURES EXPOSED TO VARIOUS TREATMENTS

Experiment no.			4		5	
Duration			6 days		5 days	
Fungus: *Sclerotinia americana*			6 days old		7 days old	
Treatment			Air passed through	Standing in open dish	Air passed through	Standing in open dish
			Per cent and type of germination			
Distilled water, bathing sprayed surfaces	Bordeaux mixture made with distilled water	Filtrate 1	87.3 A–	87.6 B+	12.4 D	91.0 C
		Filtrate 3	96.6 A	96.7 A–	90.5 A–	97.3 A
		Sprayed slides	0	....	0	....
	Bordeaux mixture made with rain water	Filtrate 1	96.7 A	37.7 C	94.3 A	96.8 B
		Filtrate 3	98.0 A	98.5 A	99.0 A	98.4 A
		Sprayed slides	0	....	0	....
Rain water, bathing sprayed surfaces	Bordeaux mixture made with distilled water	Filtrate 1	0	96.4 A–	21.6 D	63.5 B+
		Filtrate 3	39.3 C	....	97.1 A	96.5 A
		Sprayed slides	0	....	0	....
	Bordeaux mixture made with rain water	Filtrate 1	0	46.5 B–	5.3 D	58.8 B–
		Filtrate 3	29.4 D	56.2 B	97.9 A	93.7 A
		Sprayed slides	0	....	0	....
Controls		Distilled water	98.5 A		98.8 A	
		Rain water	89.8 B+		95.7 A	

Discussion. The results presented in table 9 show that the continued passage of air through distilled water standing on bordeaux mixture or copper-lime does not promote the solution of copper any more than where the water is left undisturbed. The lower percentages of germination obtained in experiment 3 cannot be explained.

A study of the results given in table 10 shows that rain water does not allow as high a percentage of germination of the spores of *Sclerotinia americana* as does distilled water. No explanation of this can be given. This difference is evidenced, however, in both the experiments. The distilled water in these experiments shows a little more solvent action than did that in the previous ones. However, the difference is but small. The rain water appears to possess a greater solvent power than does the distilled water.

It should be noted that, in all cases, spores placed directly on the sprayed slides were completely inhibited from germination. These slides had been exposed in water to the action of the air for periods of from 5 to 9 days. This bears out a previous conclusion that the presence of the insoluble copper protectant is sufficient to inhibit germination, and shows that the copper is not appreciably leached out by long exposure to wetting with well-aerated water.

A second conclusion to be drawn from these experiments is that distilled water, either standing undisturbed or receiving a continuous passage of air, does not promote the solution of copper to an effective extent.

Thirdly, rain water exhibits greater solvent action than does distilled water. It is difficult to determine how much of this apparent toxicity is actually due to the solvent action of the rain water, for the germination is also slightly reduced in pure rain water. In any case, the results of the experiments show that sufficient copper is not brought into solution by this means to more than slightly inhibit the germination of the spores. This is especially significant when it is realized that here a very large amount of carbon dioxide came in contact with the insoluble copper for long periods of time. It is believed, therefore, that under natural conditions atmospheric agencies cannot bring about the liberation of copper necessary to kill or to effectively inhibit the germination of spores.

Spore excretions

The solvent action of filtrates from suspensions of germinating spores

Hypothesis. The hypothesis considered in the following paragraphs is essentially that of Wallace, Blodgett, and Hesler (1911:186), namely, " that the germinating spore secretes a dissolving agent which acts on the insoluble fungicide to bring small quantities of the same into solution "; that is, water in which spores have germinated possesses dissolving powers greater than that of pure water.

Wallace, Blodgett, and Hesler (1911) performed a few preliminary experiments with lime-sulfur. They placed spores of *Sclerotinia cinerea*[2] in water in a watch glass, allowed them to germinate for three days, and then filtered the suspension. This filtrate they considered to consist of water plus the hypothetical dissolving agent secreted by the spores. They report (page 187 of reference cited):

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[2] Presumably *S. americana.*

> Some drops of the filtrate were placed on slides sprayed with lime-sulfur 1-25 and some on clean slides. Germination tests in water in which no spores had previously germinated were carried through the entire experiment as a check. All slides were kept in moist chambers for two days, then the water from each slide was removed to clean ones, and spores added.

The germination results were as follows: control, 40 per cent; water from sprayed slide, 8 per cent; water plus spore filtrate from clean slide, 25 per cent; and water plus spore filtrate from sprayed slide, 0 per cent. A near duplicate of this experiment gave somewhat similar results.

Experiments. The method employed by the author was in principle similar to that of Wallace, Blodgett, and Hesler. In some preliminary experiments the results were not satisfactory, largely because of an apparent staling effect of the water in which the spores had germinated. The spores employed here were those of *Botrytis allii.* In all later tests, conidia of *Sclerotinia americana* were used and excellent results were obtained. This modification, together with a greater familiarity with the method, resulted in the elimination of the staling-effect factor.

The fungicides used were colloidal bordeaux membranes from 4-4-50 bordeaux mixture, and copper-lime dust. The colloidal membranes were separated from the mixture by centrifuging. The clear limewater and lime particles were thus eliminated. Neither the original bordeaux mixture nor the bordeaux membranes showed any free copper by the potassium-ferrocyanide test. The bordeaux membranes were sprayed into petri dishes and allowed to dry for 12 hours. With the copper-lime dust, the petri dishes were first sprayed with distilled water, and then the dust was applied and allowed to dry likewise.

Spores of *Sclerotinia americana* were placed in distilled water in open petri dishes and allowed to germinate for 12 hours. The germination period was thus reduced because in nature spores would not normally germinate for as long periods as two and three days before fungicidal action would become operative. In all cases the germination was uniformly about 97 per cent. These suspensions were then filtered, and the resulting solution constituted the "old spore filtrate." Ten cubic centimeters of this was added to each of a number of petri dishes, in which the bordeaux membranes or copper-lime dust had been placed previously. At the same time, ten cubic centimeters of distilled water was placed in an equal number of petri dishes, likewise coated with bordeaux membranes or copper-lime dust. The remainder of the old spore filtrate was stored in a Pyrex beaker. All preparations were allowed to stand exposed to the air for 36 hours, and then filtered.

Twelve hours previous to this filtering, a fresh spore suspension had been prepared and allowed to germinate for 12 hours. This suspension was then ready to be filtered at the same time as those described above. It constituted the "new spore filtrate." The second spore filtrate was prepared in order that possible changes occurring in the old spore filtrate during the period of standing might be checked.

The solutions after filtering were thus from (I) old spore filtrate, (II) new spore filtrate, (III) distilled water on fungicide, and (IV) old spore filtrate on fungicide. To test these solutions for toxicity, spore-germination tests were made with conidia of *Sclerotinia americana*. However, in order to have comparative tests — that is, to balance the possible staling effects of the spore filtrates — equal volumes of the spore filtrates were added to equal volumes of solution III. This necessitated adding an equal volume of distilled water to solution IV, so that the respective dilutions would be approximately equal.

The final solutions in which the spore-germination tests were then made, consisted of (1) control, distilled water, (2) old spore filtrate, (3) new spore filtrate, (4) solution III plus old spore filtrate, (5) solution III plus new spore filtrate, and (6) solution IV plus distilled water.

The germination tests were made in the usual manner. In general, nos. 1, 2, and 3 were carried in duplicate, 4 and 5 in triplicate, and 6 in quadruplicate, moist-chamber tests. In this manner six separate experiments were made with bordeaux membranes and five with copper-lime dust. The results appear, respectively, in tables 11 and 12.

TABLE 11. Solvent Action on Bordeaux Membranes of Filtrates from Suspensions of Germinating Spores: Percentage Germination of Sclerotinia americana Conidia in the Filtrates

	Experiment no.					
	1	2	3	4	5	6
	Per cent and type of germination					
Old spore filtrate	......	96.2 A	96.6 A	98.0 A	96.2 A	98.0 A
New spore filtrate	62.0 C	98.2 A	97.7 A	98.6 A	96.4 A	98.1 A
Filtrate from water on fungicide; plus old spore filtrate	......	97.6 A	95.3 A	98.1 A	37.3 B	97.9 A
Filtrate from water on fungicide; plus new spore filtrate	88.0 A	98.4 A	97.6 A	76.2 A	37.8 B	98.1 A
Filtrate from spore filtrate on fungicide; plus distilled water	11.6 C	56.4 C	3.8 D	8.9 C	7.4 C	20.8 B
Control	96.5 B	98.2 A	97.7 A	98.6 A	91.2 A	97.9 A
Age of spores, in days, for:						
Old filtrate	5	6	6	6	8	8
New filtrate	7	7	7	7	9	9
Control	8	8	8	8	10	10

TABLE 12. SOLVENT ACTION ON COPPER-LIME DUST OF FILTRATES FROM SUSPENSIONS OF GERMINATING SPORES: PERCENTAGE GERMINATION OF SCLEROTINIA AMERICANA CONIDIA IN THE FILTRATES

	Experiment no.				
	1	2	3	4	5
	Per cent and type of germination				
Old spore filtrate	98.5 A	95.7 A	98.3 A	98.3 A	98.3 A
New spore filtrate	98.7 A	98.2 A	98.3 A	98.3 A	98.3 A
Filtrate from water on fungicide; plus old spore filtrate	97.8 A	95.1 A	98.0 A	98.3 A	98.4 A
Filtrate from water on fungicide; plus new spore filtrate	98.6 A	98.1 A	98.2 A	72.6 A	98.4 A
Filtrate from spore filtrate on fungicide; plus distilled water	15.9 D	35.0 C	12.2 C	2.4 D	34.0 C
Control	98.5 A	97.2 A	97.0 A	97.0 A	98.5 A
Age of spores, in days, for:					
Old filtrate	8	7	7	7	7
New filtrate	9	8	8	8	8
Control	10	9	4	4	5

Discussion. The results of these repeated experiments with bordeaux mixture and copper-lime dust lead to but one conclusion, namely, that excretions from germinating spores are active in bringing the copper into solution from the insoluble fungicide.

Although the amount of copper rendered soluble in these experiments was not sufficient to entirely inhibit the germination of the spores, it must be borne in mind that here both the solvent principle and the soluble copper were doubtless greatly diluted. In nature the reaction of single isolated spores possibly differs, in that there is perhaps a greater concentration of soluble copper about the spore.

In these experiments the "spore filtrate," or filtrate from the solution in which the spores had germinated, was not found to be toxic to the spores even after standing for two days. This, therefore, cannot be a factor in the toxicity of the solutions. Likewise, as was previously shown, the action of atmospheric agencies on these copper protectants while in contact with distilled water was not sufficient to liberate any appreciable quantity of soluble copper.

From these experiments it cannot be determined at what period of germination, if any, the production of the excreted solvents by the spore is most abundant. It is perhaps possible that a spore, even in its normal life processes before germination has begun, may excrete dissolving materials.

However, regardless of these possibilities, these experiments seem to demonstrate definitely, at least on the part of germinating spores, an excreted solvent capable of dissolving copper from "insoluble" copper protectants.

The action of spore excretions on bordeaux mixture in dialyzing sacs

Hypothesis. If bordeaux mixture containing no free copper is placed in a suitable dialyzing membrane immersed in water, it may be assumed that the colloidal bordeaux membranes will not be able to diffuse outward. The dialyzing membrane, however, should allow the outward and inward diffusions of materials in solution. A solvent action on the part of the spore excretions being assumed, then, if the fungus spores are placed in water on the outside of the dialyzing membrane, the excretions will pass through the dialyzing membrane. Here reaction will take place with the bordeaux membranes, and by reason of the solvent powers of the spore excretions some copper will be brought into solution. This soluble copper will diffuse outward and react with the fungus spores, inhibiting their germination and ultimately killing them.

Experiments. Collodion dialyzing sacs were prepared according to the method of Eggerth (1921), d'Herelle (1926), and Mulvania (1926). The porosity of these sacs varies according to the ratio of alcohol and ether; a greater degree of porosity is obtained by increasing the percentage of alcohol. The first collodion sacs prepared were of the formula 23-77-1.5; that is, 23 cubic centimeters of absolute ethyl alcohol, 77 cubic centimeters of ethyl ether, and 1.5 grams of soluble nitrated cotton. The sacs were molded on the inside of 1.5x11-centimeter test tubes.

Bordeaux mixture (4-4-50) was prepared and tested for free copper. None was found. The bordeaux was then placed inside the collodion dialyzing sacs. The mouths of the sacs were tied with string and sealed with collodion. After this the sacs were thoroughly rinsed in distilled water and then placed in open petri dishes. Fresh distilled water was added to the dishes so that the sacs were just submerged. Two sacs were placed in each dish. The dishes were then divided into two lots. To one there was added a concentrated suspension of conidia of *Sclerotinia americana.* The other remained standing without spores. After two to three days the spores of the first lot were examined for germination. At this time the bordeaux-containing sacs were removed from the second lot of dishes and a suspension of spores was added to the remaining liquid. After standing thus for another two days, these spores were in turn examined for germination. This was to test for any possible outward diffusion of copper. Controls were carried throughout, in which a suspension of the spores had been placed in dishes containing dialyzing sacs holding distilled water only. In this manner three experiments were performed in duplicate. The germination results are given in table 13.

TABLE 13. PERCENTAGE GERMINATION OF SCLEROTINIA AMERICANA CONIDIA IN WATER IN CONTACT WITH DIALYZING SACS CONTAINING BORDEAUX MIXTURE

Experiment no.	1		2		3	
Collodion solution	23–77–1.5		23–77–1.5		25–75–2.5	
	Per cent and type of germination					
Spores on outside of dialyzing sacs containing bordeaux	0	0	0	0	0	0
Solution in which dialyzing sacs of bordeaux had stood	95.5 A	98.0 A	98.3 A	98.6 A	89.0 A–	89.0 A–
Control	99.7 A		98.7 A		95.0 A	
Age of spores	4 days		8 days		12 days	

These experiments were further elaborated. An effort was made to determine the effect of dialyzing sacs of varying permeability — 20–80–2.5, 30–70–2.5, and 40–60–2.5 — and the experiments were run in triplicate with each type. To obviate any complications due to the presence of excess lime, 4–4–50 bordeaux was prepared and then centrifuged. The centrifuging made possible the separation of the clear limewater and lime particles from the jelly-like bordeaux membranes. Water was added to the bordeaux to bring it back to the original strength. The mixture was chemically tested for free copper, and none was found. The mixture was then placed in the collodion sacs, and these were tied and were sealed with agar. The sacs were placed in pairs in petri dishes containing distilled water, and allowed to stand for two days. The water was then pipetted off and germination tests were made in it. These results are shown in the first column of percentages in table 14. A spore suspension of *Sclerotinia americana* was then added to the water surrounding the collodion sacs in the petri dishes. The germination was observed after two days, and is recorded in the second percentage column of table 14. In order to demonstrate whether any free copper was left in solution — that is, whether more copper had been dissolved than was taken up by the spores — the liquid surrounding the sacs was filtered and germination tests were made in the filtrate. These results are given in the third percentage column of table 14. Finally, to find whether the collodion sacs had leaked, the sacs were carefully rinsed in distilled water, placed in fresh distilled water, and allowed to stand for five days. The water was then pipetted off and germination tests were made in it. The results of this final test are recorded in the last column of table 14.

TABLE 14. Percentage Germination of Sclerotinia americana Conidia in Water in Contact with Dialyzing Sacs of Varying Permeability Containing Colloidal Bordeaux Membranes

Treatment	Set no.	Water from dishes holding sacs containing bordeaux	Spores placed in water surrounding sacs of bordeaux	Filtrates from the aqueous suspension of percentage column 2	Bordeaux sacs from percentage column 2 washed, and placed in fresh water for 5 days: water from same
		Per cent and type of germination			
Collodion 20–80–2.5	1	98.8 A	92.7 A	95.1 A	96.0 A
	2	95.8 A	91.3 A	96.4 A	96.0 A
	3	97.1 A	93.4 A	92.0 A	......
Collodion 30–70–2.5	4	96.2 A	86.1 B+	94.4 A	95.7 A
	5	97.0 A	88.3 B+	91.8 A	......
	6	97.0 A	89.4 A–	97.1 A	95.6 A
Collodion 40–60–2.5	7	85.5 A	31.4 C	97.0 A	96.6 A
	8	95.4 A	39.4 C	95.7 A	86.0 A
	9	93.4 A	38.6 C	93.4 A–	......
Control	..	95.2 A	97.5 A	97.4 A	96.6 A
Age of spores..................	..	10 days	10 days	5 days	9 days

Discussion. The results of the first three experiments appear to clearly substantiate the hypothesis of a solvent action on the part of the spore execretions toward the insoluble copper compound.

The results of the fourth experiment do not demonstrate this so clearly. The figures in percentage columns 1 and 4 of table 14 show that the collodion sacs were entire and did not leak. Hence, when they were standing in pure water there was no appreciable outward diffusion of copper. This would imply that no copper comes into solution merely on standing. The results recorded in percentage column 3 show that all of the copper which may have been brought into solution was absorbed, adsorbed, or otherwise removed from solution by the spores, or possibly also by the process of filtering.

The figures in percentage column 2 show that collodion sacs of 20–80–2.5 formula are practically impervious to the inward diffusion of any solvent, or the outward diffusion of copper, or both. The collodion sacs of 30–70–2.5 formula permitted a slight outward diffusion of copper, while the most permeable sacs, 40–60–2.5, permitted a considerable outward diffusion of copper. By a comparison with the three former experiments (table 13), it would be expected that with the collodion sacs of greater porosity — that is, 30–70–2.5 and 40–60–2.5 — sufficient copper would be allowed to diffuse outward to completely inhibit germination of the spores. It is beyond the scope of this paper to attempt to explain this difference in diffusion. However, it does appear possible that the difference in the alkalinity of the two types of bordeaux may have been a contributing factor. So far as is known, all other factors were the same.

The results of this series of experiments are believed to point definitely to a solvent action exercised by some excretion from the fungus spores.

The sphere of influence of spore excretions

Hypothesis. It is assumed that actual contact of the spore with the colloidal particles is not necessary in order that the copper be rendered soluble.

In the preceding study there was definite indication that this is the case, and an effort has accordingly been made to determine how great the separation between the spore and the copper particle may be before a lessening of toxicity is evident; in other words, to determine the sphere of activity of the solvent spore excretions, and hence of the liberated copper particles. It will be recalled that Lutman (1916) observed that no spores germinated when within a radius of one millimeter from a drop of Pickering's bordeaux. On the other hand, Barker and Gimingham (1911, 1913, 1914 b) have maintained that actual contact, or at least very close association, is necessary for toxic action.

Experiments. In a preliminary experiment, bordeaux mixture 4–4–50 was prepared which showed no free copper by the potassium-ferrocyanide test. The bordeaux was sprayed into four watch glasses and allowed to dry for 24 hours. Ten cubic centimeters of distilled water was then added to each glass, and the whole was allowed to stand thus exposed for 60 hours. In the meantime, fresh bordeaux mixture containing no free copper was placed in the form of drops on clean glass slides. Two drops were placed on each slide, each drop about 1.5 centimeters from the end of the slide. These drops were allowed to dry down for 24 hours. The end of this period coincided with the end of the 60-hours period referred to above.

A spore suspension of *Sclerotinia americana* conidia was prepared and two drops were placed on each of a number of clean glass slides. Two drops were placed also on each of the slides holding the dried drops or areas of bordeaux mixture. The drops of spore suspension and of bordeaux were separated by a space of from 1 to 5 millimeters.

The spores were allowed to settle, and thus to adhere to the surface of the slides. This required about half an hour. Then, to the slides with the bordeaux and spore-suspension areas, distilled water was added by means of a pipette, to form a film covering and connecting each pair of areas — bordeaux and spores. Thus, although the bordeaux and the spores were connected by a bridge of water, there was no direct contact between them.

The water standing in the watch glasses sprayed with bordeaux was filtered, and the filtrate was added to the clean slides holding the spore-suspension drops, so that it formed a covering film. This film resembled that of the distilled water on the other set of slides. This set constituted a check on the copper that might come into solution from the bordeaux through atmospheric agencies or other means.

A control was maintained, in which films of distilled water were placed over drops of spore suspension. All slides were placed in moist chambers and the usual germination tests were made. The usual type of control also was carried.

The germination results after 24 hours are recorded in table 15.

TABLE 15. Sphere of Influence of Spore Excretions: Percentage Germination of Sclerotinia americana Conidia

Treatment	Per cent and type of germination
Bordeaux filtrate on spores	58.2 B
Bordeaux areas near spore areas, distilled water over all	0
Water-film control	96.5 A
Control, usual (spores 6 days old)	97.8 A

The reduced germination in the case of the filtrate is doubtless due in large part to soluble calcium compounds. The ungerminated spores here appeared normal, which is characteristic of spores killed by $Ca(OH)_2$ and $CaSO_4$. However, the spores that were placed near the areas of bordeaux mixture showed a very evident precipitation of their contents, a condition characteristic of copper poisoning.

This experiment was elaborated in some detail. Drops of bordeaux mixture (no free copper) were placed, one each, on a number of slides and allowed to dry down. Then to one slide was added a drop of *Sclerotinia americana* spore suspension, and an additional second drop twice as far from the edge of the bordeaux as the first drop. On a second slide there were placed three drops of the spore suspension, on a third slide four drops, and on a fourth slide five drops. A slide with five drops is diagrammatically illustrated in figure 4. This series was made up in quadruplicate. The spores were allowed time in which to settle and adhere to the glass. The average distance between the edge of the bordeaux area and the edge of the nearest spore-suspension drop was 7.5 millimeters. Drop no. 2 was 15 millimeters distant, no. 3 was 30 millimeters, no. 4 was 40 millimeters, and no. 5 was 50 millimeters. Regardless of the number of spore-suspension drops on each slide, these distances were maintained. The number of spore-suspension drops was varied in order to further study the correlation between total toxic substance and number of spores, which has already been discussed in the copper-toxicity studies.

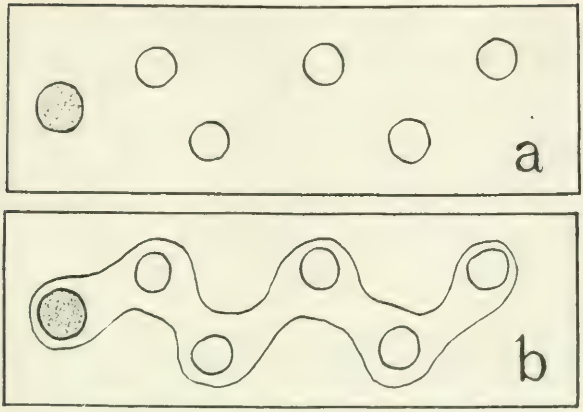

FIGURE 4. SPHERE OF INFLUENCE OF SPORE EXCRETIONS
a, A slide with a dried drop of bordeaux mixture and five drops of spore suspension. b, The same, with the drops connected by a film or bridge of water

To increase the distance and the number of spores, petri dishes were employed. A drop of bordeaux mixture was placed near the edge of a dish. On an arc of a circle 2 centimeters distant, three drops of spore suspension were pipetted, each drop as far away from the others as possible. In addition, three drops were placed on three other arcs, which were respectively 4, 6, and 8 centimeters distant from the bordeaux area. The spores were given time to adhere to the glass. This series also was carried in quadruplicate.

The drops on the slides were connected by a film of water, as was done in the preliminary experiment. With the petri dishes, the whole was flooded with a thin film of water. Germination tests were made in the usual manner, and the average results for each series are recorded respectively in tables 16 and 17. A control similar to that in the preliminary

TABLE 16. PERCENTAGE GERMINATION OF SCLEROTINIA AMERICANA CONIDIA AT VARYING DISTANCES FROM AN AREA OF DRIED BORDEAUX MIXTURE (SPORE-SUSPENSION DROPS AND BORDEAUX DROP PLACED ON SLIDES AND CONNECTED BY A BRIDGE OF WATER)

	Distance of spore-suspension drops from bordeaux area				
	7.5 mm.	15 mm.	30 mm.	40 mm.	50 mm.
	Per cent and type of germination				
2 spore-suspension drops per slide	0	0	....	......	......
3 spore-suspension drops per slide	0	13.8 D	36.6 C+	......	......
4 spore-suspension drops per slide	0	15.3 D	34.5 C+	53.0 B	......
5 spore-suspension drops per slide	0	15.4 D	41.0 B−	61.6 B+	71.8 A
Bordeaux filtrate	66.2 A				
Control	94.6 A				

TABLE 17. Percentage Germination of Sclerotinia americana Conidia at Varying Distances from an Area of Dried Bordeaux Mixture (Spore-Suspension Drops and Bordeaux Drop Placed in a Petri Dish and the Whole Covered by a Film of Water)

Distance of spores from bordeaux area				Control
2 cm.	4 cm.	6 cm.	8 cm.	
Per cent and type of germination				
79.6 C	87.8 B–	89.9 B+	90.0 A–	94.1 A

experiment was carried, in which the filtrate from water standing on a surface sprayed with bordeaux mixture was tested. The age of the spores was 6 days.

Discussion. The results from the tests of the spores placed on the slides at varying distances from the bordeaux mixture, are very interesting. It can be seen that as the distance from the bordeaux and the number of spores are increased, so the percentage and the quality of germination increase. With the petri dishes, the volume of water and the relative distances are sufficiently great so that the differences in percentage and quality of germination are not so pronounced.

Undoubtedly the filtrate from water standing in bordeaux mixture is slightly toxic. However, as has already been pointed out, it is believed that this toxicity is due to certain soluble calcium compounds present, and perhaps also to the alkalinity of the filtrates. A point that seems to substantiate this is the apparent normal appearance of the contents of ungerminated spores, which is characteristic of inhibition by calcium. Spores killed or inhibited from germination by copper show a very decided precipitation of their contents.

All of these experimental results demonstrate most clearly the fact that direct contact is not necessary in the toxic action of bordeaux mixture on spores. It is evident that the spores may be killed, or at least inhibited from germination, while at some considerable distance from the particles of bordeaux. A factor influencing this toxicity is the number of spores present. It is possible, either that the solvent spore excretions can operate over a wide area, acting on bordeaux particles to liberate copper by which the spores themselves are ultimately killed, or that the cumulative toxic action may account for their death. Or, again, both conditions may bring about this result. It seems difficult to conceive of such minute traces of copper — as required by the cumulative-action hypothesis — diffusing at such a rate as to inhibit the germination of the distant spores.

Cumulative action

The abstraction, by spores, of copper from large volumes of water flowing over copper protectants

Hypothesis. The protective action in copper fungicides, especially bordeaux mixture, may be dependent on a cumulative action of the copper in equilibrium in solution. That is, bordeaux mixture may contain always in solution a minute trace of copper infinitely beyond detection by ordinary chemical tests or even spore tests. In the presence of spores or other reactive bodies, this soluble copper is absorbed or otherwise removed from solution. An equilibrium having existed between the insoluble bordeaux and the soluble copper, this is now disturbed. Therefore, more copper will enter into solution to maintain the equilibrium. The spores will in turn remove this copper, and so on until finally the spores will have accumulated sufficient copper to ultimately destroy themselves. This, of course, assumes that the source of soluble copper, the bordeaux particles, is always present.

Experiments. In a study of this hypothesis, some preliminary experiments were performed. It was thought that if this hypothesis were true, then large quantities of water slowly dripped over " insoluble " copper protectants and then over the spores not in contact with the fungicides would carry traces of soluble copper to the spores. Because of the constantly changing equilibrium conditions about the fungicide, fresh copper would continually enter into solution and the spores would abstract enough copper to eventually become poisoned. If, however, the excretions of spores are the only solvent agent, then the spores would not be killed. So if the water were first dripped over spores capable of excreting the solvent agent, and then the water plus this agent were passed over the fungicide, sufficient copper should be brought into solution to kill the test spores.

An apparatus was accordingly devised and set up. Tests were made with spores of *Sclerotinia americana* and bordeaux mixture 4–4–50, Woburn bordeaux, and copper-lime dust. Since the experiment was, in general, unsatisfactory, a description of the apparatus is considered unnecessary. The chief difficulties encountered were the poor germination of the spores in the control apparatus, the rapid disorganization of the Woburn bordeaux, precipitating black copper oxide — which occurred also, though to a less extent, in the bordeaux mixture and the copper-lime — and the precipitation of calcium compounds from the bordeaux mixture and the copper-lime.

The results of three typical experiments are given in table 18.

TABLE 18. Percentage Germination of Sclerotinia americana Conidia in Flowing Water Which Had Previously Been Dripped over Copper Protectants

	Experiment no.		
	2	4	5
Fungicide	Woburn bordeaux	Bordeaux 4–4–50	Copper-lime
	Per cent and type of germination		
Apparatus control	55.8 B–	90.7 C	78.7 B–
Water dripped over germinating spores	41.2 B–	70.9 C–	61.6 B–
Water dripped over fungicide	0	0	17.2 D
Water dripped over germinating spores, then over fungicide	0	0	0
Control (usual)	97.5 A	97.6 A	97.8 A

Discussion. No conclusions can be drawn from these experiments because of the instability of the fungicides, which introduced new and unknown toxic factors. With the copper-lime dust there is slight evidence of a solvent action on the part of the germinating spores.

The rate of solution of soluble and "insoluble" copper compounds

Hypothesis. If suspensions of soluble and "insoluble" copper compounds are prepared in various dilutions in a parallel series, the soluble copper compounds should be immediately toxic to the extent of their total copper content. On the other hand, according to the cumulative-action hypothesis only a very small trace of the "insoluble" copper compound is at any one time in solution. It is only after a period of time that an appreciable amount of the copper can be brought into solution, and this is dependent on some reactive body being present to remove it as fast as it comes into solution. Presumably, complete solution and removal of all the copper from the "insoluble" compound would require considerable, if not almost infinite, time. Therefore, with two dilutions of the same total copper content, that of the soluble copper compound should be very much more toxic than that of the "insoluble" compound, for with the latter a period of time must elapse before any appreciable percentage of the total copper can have come into solution. If, however, spore-excretion solvents are active, there should be little difference in toxicity, though probably the soluble compound would be somewhat more toxic because of its immediate action.

Experiments. Dilute solutions of copper sulfate were prepared. Suspensions of an "insoluble" proprietary copper dust, of equal total copper content, were likewise prepared. Spores of *Sclerotinia americana* were added to these solutions and suspensions, and germination tests were made. The recorded germinations are shown in table 19.

TABLE 19. PERCENTAGE GERMINATION OF SCLEROTINIA AMERICANA CONIDIA IN SOLUTIONS OF COPPER SULFATE AND SUSPENSIONS OF AN "INSOLUBLE" COPPER DUST

Milligrams of copper per liter of solution	Experiment 1		Experiment 2	
	Soluble $CuSO_4$	"Insoluble" Cu dust	Soluble $CuSO_4$	"Insoluble" Cu dust
	Per cent and type of germination			
20.0	0	0	0	0
10.0	0	0	0	0
2.0	Trace	0.6 D+	Trace	0.6 D
1.0	2.2 D	14.6 D+	1.5 D	17.3 C–
0.2	86.2 A–	96.5 A–	80.7 B	87.5 B+
0.1	97.4 A	97.6 A	96.0 A	96.8 A
Control	97.7 A		95.5 B+	
Age of spores	5 days		12 days	

A similar experiment was performed with copper sulfate and insoluble copper phenyl acetylide (CuC_8H_5).[3] Spores of *Macrosporium sarcinaeforme* were used. The results appear in table 20.

TABLE 20. PERCENTAGE GERMINATION OF MACROSPORIUM SARCINAEFORME SPORES IN SOLUTIONS OF COPPER SULFATE AND SUSPENSIONS OF INSOLUBLE COPPER PHENYL ACETYLIDE

Milligrams of copper per liter of solution	Soluble $CuSO_4$	Insoluble CuC_8H_5
	Per cent of germination	
10,000.0	0	0
1,000.0	0	0
100.0	0	91.6
10.0	Trace	97.0
1.0	2.8	97.2
0.1	96.3	97.1
Control	97.0	97.0

Discussion. Following the reasoning advanced above, the results shown in table 19 seem to point clearly to a solvent action on the part of the fungus spores. However, it is possible also that any cumulative action may be masked by that of the spore-excretion solvent.

In table 20, spore-solvent action appears to be much less marked. This perhaps may not be unexpected, since copper phenyl acetylide is not ordinarily used as a copper protectant and hence need not necessarily be expected to possess protectant properties. On the other hand, there is a lack of evidence in support of the cumulative-action hypothesis.

In conclusion it may be stated that these preliminary experiments fail to give any very substantial evidence on the cumulative-action hypothesis.

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[3] The copper phenyl acetylide was kindly furnished by Professor J. R. Johnson, of the Department of Chemistry, Cornell University.

THE NATURE OF THE SPORE EXCRETIONS

In the foregoing studies, experimental evidence seems to establish the presence of an excretion from the spores which exerts a solvent action on the insoluble copper protectant, thus freeing soluble copper. It is hoped that, at some future date, investigations regarding the nature of the spore excretions may be undertaken. At this time only some preliminary studies will be mentioned.

None of the investigators who have been inclined to the spore-excretion hypothesis appear to have attempted a study of the nature of the spore excretions. In the case of germinating seeds and the roots of young plants, Barker and Gimingham (1914 a) state that carbon dioxide is an active agent. However, they submit no experimental evidence. Other workers also have suggested the possible rôle of carbon dioxide.

Attempts have been made to determine the hydrogen-ion concentration of the water in which spores were germinating. Spores of *Sclerotinia americana* were allowed to germinate in distilled water for varying periods of time. Hydrogen-ion determinations were attempted of suspensions of germinated and ungerminated spores and of filtrates from each. Two standard methods were used — that of the quinhydrone electrode, and that of indicators. The results were very unsatisfactory. No constant readings could be obtained, nor could any consistent difference between these solutions and distilled-water controls be demonstrated. Apparently these solutions are almost, if not entirely, devoid of buffer action, and accurate determinations of the hydrogen-ion concentration are impracticable if not impossible. One interesting observation was made, however, namely, that in the process of filtration the filtrate is rendered more alkaline, usually to the extent of pH 0.3 to 0.5 more than the original solution.

Following the failure to obtain satisfactory results in the hydrogen-ion determinations, some preliminary tests were made in determining total acidity by titrations. To insure a very sensitive test, the method of Rosa, Vinal, and McDaniel (1913, 1914), employing iod-eosine as the indicator, was used.[4] Five-cubic-centimeter samples of the spore-excretion water were taken, and were titrated with N/1000 NaOH. Hydrogen-ion determinations of samples of distilled water which had been exposed for the same length of time that the spores were allowed to germinate — from 16 to 28 hours — also were made. The solutions were not filtered.

The distilled-water controls required from 13/20 cubic centimeter of N/1000 NaOH through neutrality, to 4/20 cubic centimeter of N/1000 HCl, for neutralization to iodeosine. The spore-excretion solutions were found to be more acid than the water, and a fairly constant difference of about 5/20 cubic centimeter of N/1000 NaOH was observed. That is, the spore excretions had an acidity equivalent to N/20,000 HCl.

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[4] The writer wishes to acknowledge his indebtedness to Dr. A. S. McDaniel for assistance and advice in performing these tests.

Titrations were made also with the indicator brom thymol blue. The difference obtained in this case was from 4/20 to 7/20 cubic centimeter of N/1000 NaOH. The two indicators thus agree well with each other. The actual acidity of the spore excretions of *Sclerotinia americana* conidia from these preliminary tests appears to be very slight. It is possible that some weak organic acid or acids are excreted by the fungus spores, and that this condition accounts for the solvent action.

SUMMARY

1. An extensive review of the literature relative to the liberation of copper from insoluble copper protectants is presented in this paper. In the main, three hypotheses have been advanced by investigators to explain this: (1) that of atmospheric agencies; (2) that of suscept excretions; and (3) that of the dissolving action of spore excretions. A less common hypothesis is that of the cumulative action of the toxic agent in equilibrium solution. The present study is concerned primarily with investigating the hypothesis of the dissolving action of spore excretions. Some attention has been given also to the hypothesis of cumulative action.

2. The experimental procedure employed was that of testing by spore germinations, as described in the second paper of the series. In general, conidia of *Sclerotinia americana* were used.

3. The toxicity of copper-sulfate solutions toward conidia of *Sclerotinia americana* was found to be positively correlated with total copper content and negatively correlated with number of spores. Total copper content must be calculated on the basis of concentration and volume of solution. However, with increasing volumes, other factors being constant, the solutions become relatively less toxic, presumably because of the factor of diffusion. With increasing spore age, given solutions become more toxic. *Sclerotinia americana* spores 5 to 10 days old, present to the extent of about 1000 per 0.05 cubic centimeter of solution, are markedly sensitive to 0.2 milligram of copper per liter of copper-sulfate solution.

4. The toxicity of certain calcium compounds toward conidia of *Sclerotinia americana* was studied. Chemically pure calcium carbonate was found to be non-toxic, but C. P. calcium-hydroxide and C. P. calcium-sulfate solutions were somewhat toxic. Calcium-hydroxide solutions ceased to be toxic at dilutions of 0.1 milligram per liter of solution, while calcium-sulfate solutions ceased to be toxic at 0.7 milligram per liter.

5. The filtrates from C. P. copper carbonate and copper hydroxide in distilled water contained quantities of soluble copper sufficient to kill the spores of *Sclerotinia americana*.

6. The action of atmospheric agencies was investigated. In no case was copper brought into solution in toxic concentrations in distilled water which had stood on glass surfaces coated with bordeaux mixtures or copper-

lime dust. When air was forcibly drawn through the distilled water which had stood on the bordeaux mixture (dried) or the copper-lime dust for periods of 5 to 9 days, the resulting filtrates were rendered only slightly toxic. If rain water, which exhibits greater solvent action, was substituted, the filtrates were more toxic. However, rain water in itself does not allow quite as good germination of the spores of *Sclerotina americana* as does distilled water. The action of atmospheric agencies in rendering copper soluble from insoluble protectants seems to be relatively unimportant.

7. The filtrate from water in which spores of *Sclerotinia americana* had germinated, when placed on bordeaux mixture (dried) or copper-lime dust for 2 days, invariably rendered the filtrate from the resulting suspension toxic. The filtrate from suspensions of germinating spores was non-toxic. The control solutions — that is, the filtrates from pure water which had stood on the protectant — always gave a high percentage of germination. This seems to demonstrate the excretion of a solvent agent by the germinating spores.

8. Bordeaux mixture placed in collodion sacs of a certain degree of porosity, and the whole placed in distilled water, did not appear to give a diffusible soluble copper, since the outside water was non-toxic to the spores when the sacs were removed. If, however, spores were placed in the water in the presence of the bordeaux-containing sacs, they did not germinate, thus indicating the production by the spores of some solvent agent capable of diffusing through the dialyzing membrane to free diffusible copper.

9. In all the experiments performed, the copper protectants were definitely toxic only in those cases in which the spores, or filtrates from suspensions of germinating spores, were able to maintain an aqueous connection with the protectant. Spores placed at varying distances from a drop of dried bordeaux mixture, and connected therewith by a bridge or film of water, were entirely inhibited from germinating at a distance of 7.5 millimeters. As the distance of the spore from the bordeaux increased, the percentage germination of the spores also increased. This clearly demonstrates that direct contact is not essential for toxic action by bordeaux mixture or copper-lime dust.

10. No definite evidence could be obtained, either in favor of or against the cumulative-action hypothesis — that is, the accumulation of copper, by the spores, from the very minutes traces presumably present in solution in equilibrium with the "insoluble" copper protectant.

11. Preliminary tests seemed to indicate that the spore excretions of *Sclerotinia americana* were very slightly acid, to the extent of about the equivalent of N/20,000 HCl.

12. All the evidence obtained in this investigation appears to demonstrate that the chief agency in bringing about the liberation of copper

from insoluble copper protectants is spore excretions. Various atmospheric factors, especially meteoric waters, may be supplementary agents, and perhaps also the excretions from suscept parts, though this has not been clearly established. In any case, these agents appear to be nonessential for protective action. In addition, various calcium compounds in the case of bordeaux mixture and copper-lime dust, and probably also the alkalinity of the mixture, play a rôle. Finally, there may be an infinitely slight accumulation of copper from that which presumably is always present in equilibrium with the most "insoluble" of copper protectants. No definite conclusions can be drawn from the preliminary experiments performed to study this point.

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A STUDY OF MEADOW-CROP DISEASES IN NEW YORK

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A STUDY OF MEADOW-CROP DISEASES IN NEW YORK[1]

James G. Horsfall

OBJECT OF THE INVESTIGATION

A paucity of information, especially that which is easily accessible, led to an investigation of meadow-crop diseases. In studying such a wide range of diseases, the writer accumulated an extensive bibliography that has made possible the bringing together of the important information extant on the diseases of meadow crops. No claim to completeness of bibliography is made since many papers, which for various reasons were not found in indices, abstract journals, or other papers, are missing.

Briefly, the objects of the work have been: (1) to study methods of determining loss from foliage troubles, (2) to describe the diagnostic characters for the diseases, (3) to untangle the nomenclature of the causal organisms, (4) to make observations and experiments upon life history, especially the factors governing spore germination, and (5) to determine the effects of sulfur and copper dusts in combating these diseases.

The pioneer work of Kightlinger (1925) and the later work of Greaney (1928) and of others in controlling wheat rust successfully with fungicides stimulated the investigation looking toward a similar control for meadow-crop diseases. This study has centered around laboratory tests of the effect of fungicides upon germinating spores, using methods long applied in the laboratory of Plant Pathology at Cornell University and described recently by McCallan (1930). The laboratory tests were supplemented by greenhouse and field studies, although the latter was limited by the facilities available. As McCallan has pointed out, the results of laboratory tests may be used as reliable indicators of the efficiency of fungicides in controlling diseases. Laboratory tests serve admirably to narrow the scope of field work which, of course, will furnish the ultimate criterion of the value of the fungicides.

The diseases of the three clovers, red clover, *Trifolium pratense* L., alsike clover, *T. hybridum* L., and white clover, *T. repens* L., and of timothy,

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[1] Also presented to the Faculty of the Graduate School of Cornell University, February, 1929, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

Author's acknowledgment. The author wishes to express his appreciation for many helpful criticisms and suggestions to Professor H. H. Whetzel, who suggested the problem and under whose immediate direction the work was done. He wishes also to acknowledge the assistance rendered by Professor M. F. Barrus and the other members of the staff of the Department of Plant Pathology, Cornell University, who have rendered assistance of various kinds.

Phleum pratense L., have been studied most intensively, and some attention has been paid to the diseases of the less-important clovers and grasses in the State. Some consideration has been given to a destructive disease of blue-grass, *Poa pratensis* L., which, though not a crop of first rank in the State, is commonly found on lawns, golf courses, and in meadow mixtures. Many pathogenes attacking the first-named suscepts have been studied also in their relation to other possible suscepts. The diseases of alfalfa, *Medicago sativa* L., were included in the survey of the State, but a further study of them was not made unless the plant happened to be a suscept of some of the other diseases involved in the investigation. The following is a list of the other suscepts mentioned in this paper: crimson clover, *Trifolium incarnatum* L.; sweet clover, *Melilotus alba* Desr.; zigzag clover, *Trifolium medium* L.; hop clover, *Trifolium agrarium* L.; vetch, *Vicia villosa* Roth.; yellow trefoil, or black medick, *Medicago lupulina* L.; red top, *Agrostis alba* L.; orchard grass, *Dactylis glomerata* L.; quack grass, *Agropyron repens* (L.) Beauv. The writer has followed Wiegand and Eames (1925) in the use of Latin names of the suscepts found in New York.

IMPORTANCE OF MEADOW CROPS IN THE STATE

An investigation of the diseases of meadow crops in New York is particularly important since hay is a valuable crop in the State and since New York leads the Union in its production. According to the *New York State Crop Report* for January, 1928, published by the New York State Department of Agriculture and Markets, the tame-hay crop of the State in 1927 was worth $82,614,000. This is more than twice the value of the potato crop, the nearest competitor, which is listed at $35,775,000. The hay crop in 1927 was worth more than the combined valuation of potatoes, corn, apples, and oats, which are the crops of next importance arranged in descending order. It was worth also twice as much as all the following crops combined: winter wheat, barley, beans, buckwheat, cabbage, grapes, pears, strawberries, and cauliflower, each one of which is valued at more than $2,000,000.

In the United States as a whole, hay ranks third in value as a crop, being exceeded only by corn and cotton. It, therefore, is obvious that an investigation of the diseases of the hay crop is not without economic significance. This is especially true in New York, which leads the Nation in hay production, both in acreage and in tonnage (Callander and others, 1927:986). In 1926, New York planted a timothy and clover mixture on 2,205,000 acres and harvested 2,999,000 tons of hay. Her nearest rival in hay production is Wisconsin, where the 1,710,000 acres planted in 1926 yielded 1,650,000 tons of hay.

SURVEY OF THE MEADOW-CROP DISEASES OF THE STATE

THE NECESSITY OF A METHOD FOR DETERMINING LOSS

The necessity of having a satisfactory method for determining which are the important diseases, and for determining their distribution and their severity, has been impressed upon the writer repeatedly during the past three years. Pathologists have worried long over the problem of determining the importance, both relative and absolute, of leaf and stem diseases. Tehon (1927) has made an interesting study of losses caused by cereal diseases, but his method seems laborious. In 1928 an investigation of methods for determining the losses from meadow-crop diseases was undertaken. The writer realizes that the method described herein fails to fulfill all the requirements of perfection, but it is presented in the hope that it may be a contribution toward that end. The technic was developed as fully as the time available permitted. First, it was determined where the most hay was grown; then, during June, just prior to the first cutting, a survey trip was made through these regions, stopping frequently and determining as nearly as possible what diseases were present and how destructive they were. Thus a random sample of the meadow-crop diseases of the State was procured which served as a basis for arranging the diseases in the relative order of their importance and for calculating the losses that they bring about. During the course of the work 194 fields in 35 counties were examined. As a rule, no two stops were made within five miles of each other.

DISTRIBUTION OF MEADOW CROPS

Method of determining meadow-crop distribution

The distribution of hay crops in the State was obtained from the 1925 Agricultural Census by calculating the percentage of crop land, by counties, devoted to each kind of hay — timothy alone, timothy and clover mixed, clover alone, and alfalfa alone. Because timothy alone in a meadow results from the gradual dying out of clover in the mixture and because clover is rarely planted by itself, the percentages of crop land devoted to a mixed stand of timothy and clover, as shown in figure 1, are taken as a fair index of the distribution of cultivated hay, other than alfalfa, in the counties of the State. The survey itinerary was laid out with these points in mind. The percentage of crop land devoted to hay is somewhat misleading in some instances, a marked case being Sullivan County in the Catskill Mountains where the amount of crop land is very limited.

Method of gathering data in the field

The following method of taking data finally was adopted. It gives a fairly quantitative measure of the disease and at the same time requires

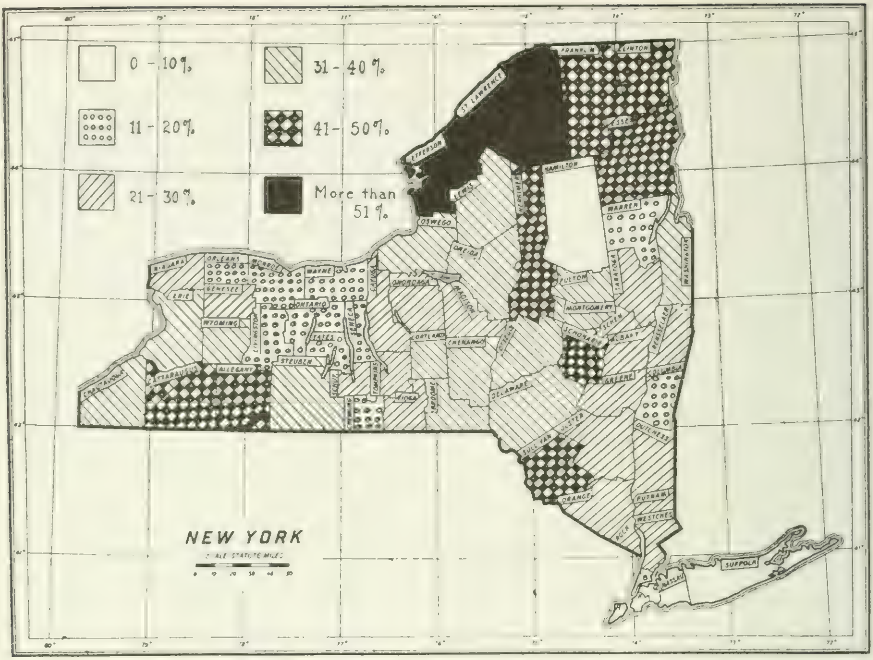


FIGURE 1. PERCENTAGE OF CROP LAND IN NEW YORK DEVOTED TO TIMOTHY AND CLOVER MIXTURE

a minimum expenditure of time in each field. It is essentially the same as that used by Köck (1927) in determining the susceptibility of apple varieties to powdery mildew.

The following classification of the amount of disease present was used: heavy, moderate, light, a trace, and no infection. The limits in the percentage of diseased leaves or stems for each group, depending upon the nature of the malady, were arbitrarily established as follows: heavy, from 65 to 100 per cent; moderate, from 25 to 65 per cent; light, from a trace to 25 per cent; trace, only a few scattered cases in a field. At first these limits were founded upon actual counts of each disease in each field, but after the groups had been used a short time, ability to estimate the percentages rather accurately was attained. The limits of each group are widely separated, to be sure, but since a great many factors, such as local rainfall, air and soil drainage, density of stand, variety, and soil types, jointly determined the amount of infection, the method probably is as useful as that of making several counts of each disease in each field.

In illustrating the method of assembling data, suppose a single suscept, red clover, be used. The data for each disease are arranged in a fraction, the " index of infection." The total number of fields of red clover observed is the denominator, which is the same for all diseases of this suscept. Each of the five groups of infection is represented by a bracket of figures in the numerator, the figures showing the percentage of cases in which the amount of infection has been placed in that group. For example, Pseudopeziza leaf spot has been found to be represented by the fraction $\frac{(3)\ (24)\ (42)\ (13)\ (18)}{119}$. From this fraction, it is readily concluded that red clover was observed in 119 fields; the disease was adjudged heavy in 3 per cent of the cases, moderate in 24 per cent, light in 42 per cent, a trace in 13 per cent, and 18 per cent of the fields showed none of it. If, therefore, the numerator be large toward the left side, the disease is severe in the State; but if large toward the right side, the disease is relatively unimportant. Unfortunately, the fractions may not necessarily be directly comparable. Anthracnose is a case in point. It has the following fraction: $\frac{(1)\ (9)\ (33)\ (15)\ (42)}{119}$. Hence it may be said to be not so serious as the Pseudopeziza leaf spot. Such a conclusion probably is fallacious in as much as the anthracnose organism attacks stems and tends to stunt or kill parts above the lesion, whereas Pseudopeziza causes only local injury to the leaves.

It became more and more evident as the work progressed that the selection of the grouping method was a happy one, for in all fields in the same group approximately the same ratio appeared to exist between the percentage of healthy leaves, the percentage of leaves in various stages of disease, and the percentage of dead leaves. If, for example, 60 per cent of the leaves were infected, then about 15 per cent of these would be dead, and the others would lie along an infection gradient from severely infected to healthy. If such a relation held true, then a method of measuring the loss, say in a field rated as moderately infected, should be applicable to the other groups as well. But to find such a method — that was more difficult.

ECONOMIC IMPORTANCE

Nature of loss

Leaf-spot diseases of meadow crops induce loss (1) by lowering the quality or palatability of the affected tissues, (2) by reducing the carbohydrate-producing area, (3) by causing defoliation during growth, and

(4) by increasing the percentage of leaves that shatter during harvest. The operation of the second and third factors mentioned results in subsequently diminished growth and a lowered quantity of sugars and other carbohydrates available for food in the hay. Stem and root diseases cause more profound injuries than do leaf-spot diseases, because they attack the supporting and conduction systems of the plants, resulting in loss of vitality, in wilting, and in stunting.

Amount of loss

Method of determining relative importance

The acquisition of an index of infection for each disease places the investigator in a position to determine the relative importance of the diseases.

Method of determining absolute loss

The index of infection was used as a basis for calculating the absolute loss caused by these leaf-spot diseases. Methods involving the measuring or weighing of plants from representative fields of each infection group were abandoned because variations in soil, stand, age of stand, age of plants, varieties, air and water drainage, as well as inaccuracy of balances with pans large enough to hold a plant, all function jointly in making such methods valueless.

It thus was essential to make all measurements in the same field and, if possible, on the same plants. The possibility of leaf measurements presented itself in this connection. A field of red clover in which about 45 per cent of the leaves were diseased, and was therefore moderately infected with *Macrosporium sarcinaeforme*, was chosen for these leaf measurements. The disease in this field seemed as nearly ideal as possible for study because (1) the clover was relatively free of other pathogenes; (2) the area of the leaf spots, which were practically circular, was easy to determine by averaging two diameters; and (3) the clover, which was growing rapidly, gave a fair amount of foliar material on each plant with which to work.

Plants picked at random five paces apart were brought to the laboratory and were placed in water. The number of dead leaves was noted, the area of each living leaf was found with a planimeter, and the spots on the leaf were classified according to size in thirteen groups ranging from 1/50 up to 13/50 inch in diameter. From these data the total leaf area, the total spotted area, and the percentage of spotted leaf area for each plant could be calculated.

The figures thus obtained are set forth in table 1.

TABLE 1. Determination of Diseased Area of Red-Clover Leaves Affected by Macrosporium Sarcinaeforme

Plant no.	Number of leaves dead	Total number of leaves	Area in living leaves (square inches)	Area spotted (square inches)	Area spotted (per cent)	Total diseased area (per cent)*
1	7	34	73.696	1.9261	2.61	22.66
2	2	9	8.416	0.1727	2.05	23.82
3	7	14	7.360	0.0345	0.46	50.23
4	19	31	17.088	0.2573	1.51	61.88
5	15	35	34.432	2.6413	7.67	47.24
6	3	13	14.784	0.2793	1.89	24.53
7	3	16	14.448	0.3479	2.41	23.66
Average	..	..	......	......	2.66	36.30

* This last column of the table, which shows the percentage of total foliar area diseased, was calculated by assuming the area of each dead leaf to be equal to the average area of the living leaves. Then the total area of dead leaves is added to the total area in spots, this is divided by the total area of all leaves, and the quotient is expressed in a percentage.

It may be argued that some leaves are killed by shading, but where no leaf spots are present, fields show almost no dead leaves even though the stand be exceedingly dense. Apparently, healthy clover leaves are very resistant to shading. In the field concerned in the measurements, however, the stand was thin enough so that shading was reduced to a minimum.

If the dead leaves and the dead spots in living leaves be charged against the pathogene and considered a total loss to the grower, then he lost approximately 36 per cent of the leaf material in that clover crop. According to Piper (1921:398), clover hay by weight consists of about 60 per cent stems, 30 per cent leaves, and 10 per cent flower heads. If this be true, then every ton contains 600 pounds of leaves. Since 36 per cent of this is destroyed (table 1), approximately 216 pounds out of every ton was lost from this field of red clover. This was a loss of material that contains about three times as much protein as do the stems. It is true that the leaf loss is not total since most of the dead material is present, but this is counterbalanced on the other hand by (1) decreased growth additions of stems, leaves, and flowers, (2) lowered quality, and (3) increased shattering during harvest. For the purposes of this paper, therefore, the assumption is made that a clover leaf-spot disease of moderate intensity reduces the leaf yield by a third, and this reduces the yield of the entire crop by about 11 per cent. Since 45 per cent of diseased leaves, an average for the moderate class, causes a total loss of 11 per cent, then each per cent of diseased leaves causes a loss of 11/45, or 0.25 per cent, in round numbers.

This calculation probably tends to be more conservative than otherwise. If the spots appear early in the life of the plant, they will cause more profound effects than if they appear late. In as much as this effect is extremely difficult to evaluate, the calculation has not been altered to account for it.

In the course of the measurement of the diseased leaves, the question arose as to the amount of affected tissue necessary to induce yellowing and death. Obviously, the reactions in each spot affect the physiology of the adjacent tissue, and hence the entire area need not actually be spotted before the leaf dies. The most severely diseased leaf measured in preparing table 1 had 19.82 per cent of its area visibly spotted; another leaf had 17.28 per cent of its area spotted. To determine whether these leaves approached the limit, a number of the most severely spotted unyellowed leaves and severely spotted incipiently yellowed leaves were collected from the same field and were measured as before. These data are presented in table 2.

TABLE 2. PERCENTAGE OF AREA OF RED-CLOVER LEAVES SPOTTED WHEN SEVERELY DISEASED BY MACROSPORIUM SARCINAEFORME

Leaf no.	Unyellowed leaves			Incipiently yellowed leaves			
	Total area (square inches)	Area spotted		Leaf no.	Total area (square inches)	Area spotted	
		Square inches	Per cent			Square inches	Per cent
1	3.920	0.7864	20.06	1	2.240	0.1862	8.31
2	2.448	0.2450	10.01	2	3.872	0.3433	8.90
3	2.448	0.3571	14.59	3	1.728	0.2039	11.80
4	2.960	0.4098	13.80	4	2.288	0.2757	12.10
5	2.368	0.2609	11.02	5	2.704	0.5102	18.90
Average	.....	......	13.85	....	.....	......	12.00

The assumptions in making these measurements are (1) that the incipiently yellowed leaves represent a more advanced stage of laminar infection than do the unyellowed ones; (2) that therefore a larger percentage of their area is spotted; and (3) that a point midway between these two extremes is the very largest percentage of area that could be diseased without killing the leaf. A glance at table 2, however, shows that these assumptions, at least in part, are false, because the unyellowed leaves show more diseased area on the blades than do those just beginning to turn yellow. The reason for the discrepancy fortunately is not far to seek, for the incipiently yellowed leaves almost invariably have lesions on their petioles. No such lesions, however, appear on leaf no. 1 in the table of the unyellowed series or on leaf no. 5 of the yellowed series. These petiolar lesions induce a general yellowed condition in the blades, causing them to die promptly, whereas blade infection alone does not induce a uniform yellowing but rather the yellowing spreads outward from the infection foci. A uniform yellowing therefore is an unsafe criterion for determining death of leaves from leaf spots. Since, however, no leaf of the 106 measured shows more than 20 per cent of its area involved, the conclusion is that when more infections occur, the leaf dies and fails to appear on the data sheet.

This conclusion is pertinent to the discussion regarding the appropriateness of the grouping method for determining the degree of severity of the disease, because it gives a reason for the apparent correlation between the number of dead leaves and the number of living leaves on each plant. Since a leaf can withstand only a certain number of infections before it dies, the number of dead leaves should vary directly with the degeree of severity of the disease. A corollary of this conclusion is that the greatest loss arises from leaves killed by the fungus and not from spots in living tissue, even though these spots appear exceedingly numerous to the observer.

Now that each percentage of diseased leaves has been shown to cause 0.25 per cent actual loss, the reduction in yield caused by clover leaf spots in New York can be calculated from the index of infection. Of course, the basic assumption in this calculation is that each organism kills the leaf when approximately 20 per cent or less of its area is involved. This is true within the limits of error of this method of calculation.

The index of infection, the percentage loss, and the loss in tons that have been figured for each leaf-spot disease of clover and alfalfa are arranged in descending order of importance and presented in table 3. The calculation of the percentage-loss column may be illustrated by Macrosporium leaf spot of red clover. The index of infection is $\frac{(0)\ (3)\ (34)\ (25)\ (38)}{119}$. The essential calculation is to find the percentage of diseased leaves, because the loss induced by each per cent is already known. The average percentage of diseased leaves in each group, as defined on page 6, is as follows: heavy, 82 per cent; moderate, 45 per cent; light, 12 per cent; trace, essentially 0 per cent; and none, 0 per cent. If, therefore, the percentage of fields falling into each group be multiplied by the average percentage of diseased leaves in that group, the product will be the percentage of diseased leaves in that group for the State, and the sum of these products will be the percentage of diseased leaves in the entire State (with respect to the disease in question). Thus (0 × 82) +(3 × 45)+(34 × 12)+(25 × 0)+(38 × 0) = 5.43 per cent of the red-clover leaves in the State diseased with Macrosporium leaf spot. Since each per cent of diseased leaves means an actual loss of 0.25 per cent, this disease diminishes the crop by 1.36 per cent.

Losses induced by diseases in meadows

According to the *New York State Crop Report* (January, 1928), 852,000 tons of clover hay, 3,532,000 tons of clover and timothy mixed, and 569,000 tons of alfalfa were produced in the State in 1927. The approximate total yield of clover for that year, found by adding half the yield of clover and timothy mixed to that of clover alone, was 2,618,000 tons.

The calculation of the loss in tons for table 3 may be illustrated by the Pseudopeziza leaf spot of red clover. A loss of 4.62 per cent means that the total yield for the State of 2,618,000 tons is only 95.38 per cent of what it would be were this disease not present. The total yield is divided by 95.38, the quotient is multiplied by 100, and the total yield is subtracted from the figure obtained, to give the loss in tons. The same method used in calculating the losses from clover diseases is applied to alfalfa diseases because it undoubtedly is better than a guess as to the loss involved.

TABLE 3. SUMMARY OF MEADOW-CROP-DISEASE RECORDS, LEGUMES

Suscept	Fungus or disease	Index of infection	Loss	
			Per cent	Tons
Red clover, 119 fields	Pseudopeziza leaf spot	(3) (24) (42) (13) (18)	4.62	126,810
	Macrosporium leaf spot	(0) (3) (34) (25) (38)	1.36	36,095
	Anthracnose *	(1) (9) (33) (15) (42)	....	......
	Mosaic †	(0) (0) (6) (07) (87)	0.24	6,298
	Pseudoplea leaf spot	(0) (0) (6) (7) (87)	0.24	6,298
	Cercospora leaf spot	(0) (0) (3) (4) (93)	0.09	2,358
	Rust	(0) (0) (3) (4) (93)	0.09	2,358
Total			6.64	180,217
Alsike clover, 102 fields	Cercospora leaf spot	(0) (2) (35) (24) (39)	1.27	......
	Rust	(0) (7) (30) (10) (53)	0.81	......
	Pseudoplea leaf spot	(0) (0) (16) (12) (72)	0.48	......
	Mosaic	(0) (1) (7) (7) (85)	0.32	......
	Sooty blotch	(0) (0) (6) (6) (88)	0.24	......
	Stagonospora leaf spot	(0) (0) (5) (5) (90)	0.15	......
Total			3.27	......
Alfalfa, 95 fields	Pseudopeziza leaf spot	(6) (22) (14) (11) (17)	5.12	30,704
	Stagonospora leaf spot	(0) (8) (38) (8) (46)	2.04	11,849
	Root knot (Diplodina)	(2) (7) (7) (3) (81)	....	......
	White spot	(1) (1) (9) (6) (83)	0.60	3,434
	Pyrenopeziza leaf spot	(1) (0) (3) (1) (95)	0.31	1,769
Total			8.07	47,756
White clover, 14 fields	Pseudoplea leaf spot ‡	(15) (7) (50) (7) (21)	5.59	......
	Stagonospora leaf spot	(0) (0) (43) (2) (55)	1.29	......
	Cercospora leaf spot ‡	(0) (0) (36) (2) (62)	1.08	......
	Rust	(0) (0) (28) (28) (44)	0.84	......
	Sooty blotch	(2) (2) (4) (2) (90)	0.76	......
Total			9.56	......

* Anthracnose is not admissible to the loss calculations which apply to leaf-spot diseases only.
† Mosaic probably is not accurately figured by this method.
‡ Apparently a new record for the State.

According to the estimates of Callander and his associates (1927:991), which are the most recently available data, the farmers of the country received an average of $14.52 a ton for clover hay and $13.52 a ton for alfalfa during 1926. Since the hay prices have not varied widely through recent years, these prices are considered satisfactory for 1928 in New York State. On this basis New York growers actually lost approximately $2,515,000 in 1928 because of disease in their clover and $645,000 on alfalfa, making a loss of approximately $3,160,000 on these two hay crops alone. This loss came from leaf spots only and does not take into consideration such diseases as anthracnose, powdery mildew, and root rots, which undoubtedly do much additional damage in the State.

From table 3 it appears that Pseudopeziza leaf spot, anthracnose, and Macrosporium leaf spot are the most serious diseases of red clover in the State. Cercospora leaf spot, rust, and Pseudoplea leaf spot attack alsike severely, while Pseudoplea leaf spot and rust seem to be worse on white clover. Among the diseases of alfalfa, Pseudopeziza leaf spot and Stagonospora leaf spot are most destructive.

The diseases observed on grasses and miscellaneous suscepts are brought together in table 4.

TABLE 4. SUMMARY OF MEADOW-CROP-DISEASE RECORDS, GRASSES

Suscept	Fungus or disease	Index of infection
Timothy *	Cercospora leaf spot	(3) (15) (44) (19) (19)
	Heterosporium leaf spot	(3) (2) (28) (36) (31)
	Ustilago striaeformis	(0) (0) (3) (4) (93)
Blue-grass, 45 fields	Helminthosporium leaf spot	(4) (18) (50) (11) (17)
	Rust	(0) (0) (6) (4) (94)
	Septoria leaf spot †	(0) (0) (4) (2) (94)
	Ustilago striaeformis	(0) (0) (4) (2) (94)
Orchard grass, 14 fields	Cercospora leaf spot †	(0) (28) (50) (2) (20)
	Cercosporella leaf spot †	(0) (0) (7) (15) (78)

* Sixty-four-fields basis for Cercospora estimate, 93-fields basis for the others.
† Apparently a new record for the State.

From table 4 it appears that timothy is most severely attacked by Cercospora leaf spot and next by Heterosporium leaf spot. Cercospora leaf spot is also destructive on orchard grass, while Helminthosporium leaf spot is the most ruinous disease affecting blue-grass.

The miscellaneous, or rare, diseases or fungi that the survey revealed on legumes and grasses are as follows, the apparently new records being marked with an asterisk (*). On red clover: * Stagonospora leaf spot, sooty blotch, * bacterial leaf spot, powdery mildew, * girdle, and * gray mold of stems caused by *Botrytis* sp.; on alsike clover: * Stagonospora leaf spot, Pseudopeziza leaf spot, and powdery mildew; on white clover: * Pseudopeziza leaf spot, * bacterial leaf spot, * powdery mildew, and mosaic; on alfalfa: yellows, downy mildew, walnut antagonism, Cercospora leaf spot, * Pseudoplea leaf spot, * Macrosporium leaf spot, and * girdle; on sweet clover: Cercospora leaf spot, Stagonospora leaf spot, * girdle, and mosaic: on vetch: *Botrytis* sp. (causing a leaf spot); on hop clover: Cercospora leaf spot; on yellow trefoil: * Cercospora leaf spot, * Stagonospora leaf spot, * Pseudoplea leaf spot, and Pseudopeziza leaf spot; on timothy: rust and *Cladosporium graminum*; on red top: * Cercospora leaf spot, *Helminthosporium triseptatum*, and stem rust; on quack grass (a meadow crop by courtesy): *Phyllachora graminis*, stem rust, *Pyrenophora tritici-repentis* (ascospore and conidial stages), * *Septoria agropyri*, and *Urocystis agropyri*; on foxtail: Piricularia leaf spot and head smut; on orchard grass: stem rust and leaf smut; on blue-grass: powdery mildew and leaf rust.

DISCUSSION OF SPECIFIC DISEASES

The diseases discussed were studied with the following points in mind: (1) to find diagnostic characters for separating them; (2) to unravel the nomenclatorial tangles encountered; (3) to study the life history of the pathogene through field work, pure cultures and inoculations; (4) to study the factors affecting incubation and infection; and (5) to determine the sensitivity of the causal organisms to various dust fungicides, by tests in the laboratory, greenhouse, and field. A discussion of the importance of each disease in the State is usually omitted, because this is indicated in tables 3 and 4. Some information gleaned from the literature on this point, however, is given in certain instances for comparison.

DISEASES OF LEGUMES

MACROSPORIUM LEAF SPOT

Suscepts

No suscept other than red clover has been found naturally affected by Macrosporium leaf spot in New York, but alsike, white clover, alfalfa, and sweet clover have been successfully inoculated by the writer and the pathogene has been reisolated. These plants showed fewer and much smaller spots than those on the red clover inoculated at the same time, indicating that they are less susceptible. Krakover's (1917:289) negative results from inoculation trials upon alsike clover, crimson clover, white clover, and alfalfa may indicate pathogenic differences among various strains of the causal organism. Alsike clover has been reported as diseased when associated with affected red clover (Bain and Essary, 1905:503). Elliott (1917:457) reports successful inoculation of white clover and alfalfa with a pure culture of Macrosporium isolated from red clover, but he reports no reisolation of his fungus. Several others also have reported red clover as susceptible to this disease in the field (Milburn and Bessey, 1915:72; Westgate, 1908:42; Gentner, 1918; Tehon and Daniels, 1925:714). Many of these writers undoubtedly have confused this disease with a closely related Macrosporium leaf spot occurring on alfalfa.

Gentner (1918) declares that Italian red clover is the variety most susceptible to this disease. Hesler (1925:93) found no appreciable differences in the susceptibility of varieties.

The disease

Many writers have referred to this disease without giving it a specific designation. Stakman (1922:248), however, called it *large leaf spot*. Since this spot is not necessarily large, the name *Macrosporium leaf spot* is proposed to set it off more definitely from other similar leaf spots, especially Pseudopeziza leaf spot with which it is most likely to be confused. Stevens and Hall (1913:390) use the term *Macrosporiose* to denote the same idea.

History and range

Cavara (1890) first discovered this disease near Pavia, Italy, in 1890. It was reported from Germany in 1897 by Tubeuf and Smith (1897:517). Probably the first specimen collected in this country was found on clover by May Varney at Manhattan, Kansas, in 1889 (Tehon and Daniels, 1925:714). The disease has been reported from England by Milburn (1915:72). The trouble occurs in the eastern United States from Maine to Louisiana. The first specimen from the vicinity of Ithaca deposited in the Cornell Plant Pathology herbarium was collected in 1915.

The most recent and complete treatise yet published dealing with this disease is that of Krakover (1917:273–328).

Symptomatology

The lesions of this disease are limited almost exclusively to the leaf blades, although they sometimes occur on stems and petioles, where they appear as minute black streaks from 1 to 3 millimeters in length. Petiolar lesions cause the leaf to yellow prematurely. Milburn (1915:72) says that the disease affects the seeds, which are shrunken and shriveled and much darker than healthy seeds. In young stages the leaf spots are minute and light brown in color, but they enlarge rapidly, coalesce, and kill the blade when approximately 20 per cent of its area is affected. The char-

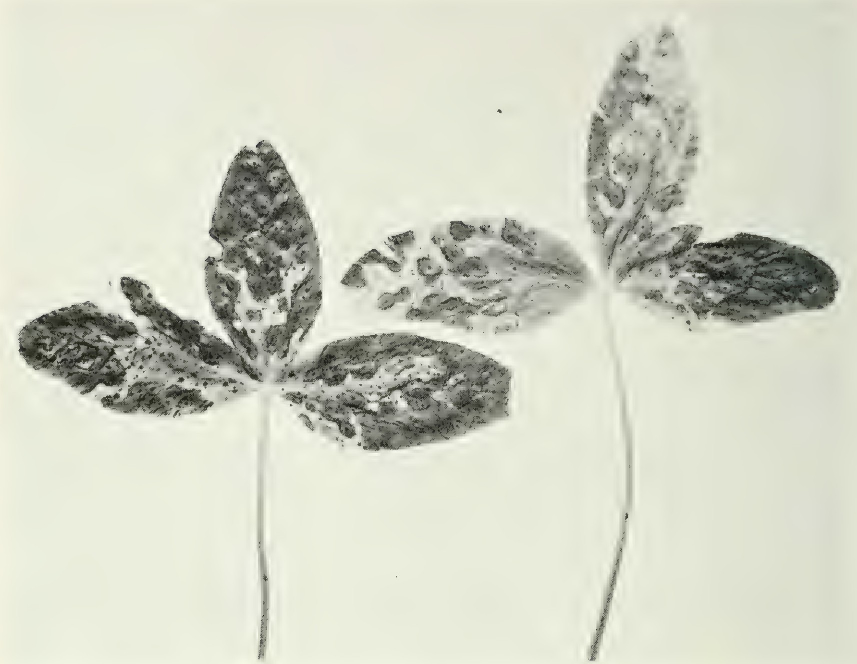


FIGURE 2. SPOTS ON LEAVES OF RED CLOVER CAUSED BY MACROSPORIUM SARCINAEFORME

acteristic concentric zones of full-sized spots, as shown in figure 2, make the disease almost unmistakable in the field. In young stages the spots are extremely difficult to distinguish from those caused by Pseudopeziza, but a careful examination usually will reveal a hint of the zonation. The older Pseudopeziza spots bear in their center the diagnostic sign of that disease, the minute dried apothecium. The latter spots are frequently dendritic in outline, but such a characteristic never occurs with the Macrosporium leaf spot.

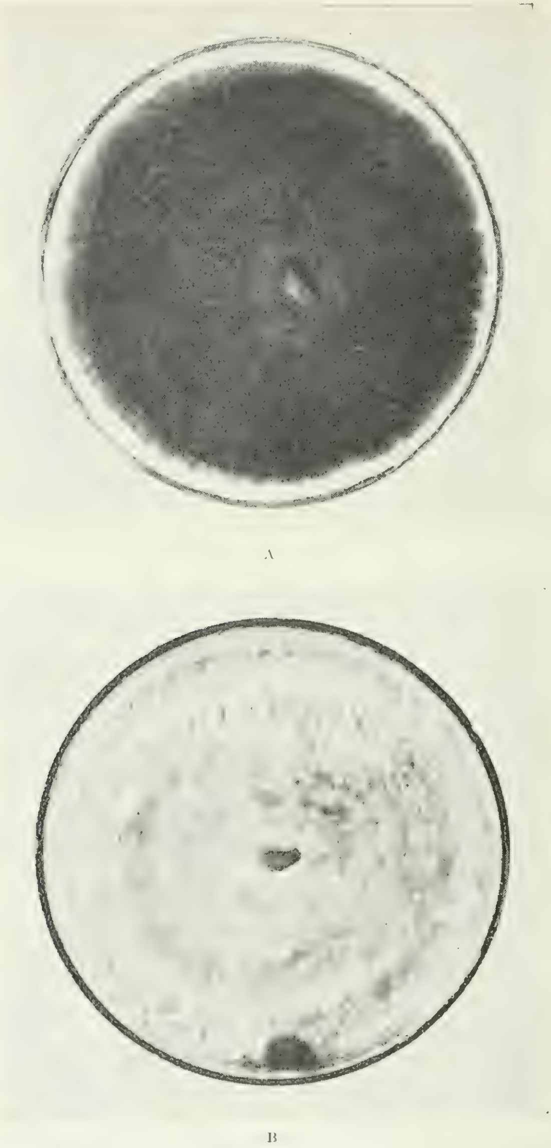
Etiology

Name, history, and classification of the pathogene. The pathogene was first described by Cavara (1890) at Pavia, Italy, as *Macrosporium sarcinaeforme*. Twenty-five conidia from one specimen collected at Ithaca measured 23.4 to 32.4 μ (mean 28.01 ± 0.026) by 19.8 to 25.2 μ (mean 23.39). The original measurements of Cavara are 24 to 28 μ by 12 to 18 μ. Krakover (1917:328) measured many spores from several different pure cultures and found that they varied from 22.4 to 37.7 μ by 19.1 to 27.4 μ. The writer's measurements fall within the latter limits.

The name of the fungus has been juggled somewhat in the literature because of a misunderstanding of the perfect stage and because of the confusion of the two fungi on clover and alfalfa. Gentner (1918) reports perithecia of *Pleospora herbarum* Rabenh. in pure cultures of a fungus which he identified as *M. sarcinaeforme.* Marchal and Marchal (1921) state that *Pleospora lycopersici* n. sp. is the perithecial form *M. sarcinaeforme.* Krakover (1917:279) concludes that the fungus on red clover is different from that on alfalfa because the conidia of alfalfa are much smaller and decidedly warty, whereas he had never seen any but smooth spores from red clover. Gentner (1918) also finds some slight morphological differences between the fungi on the two suscepts; but since conidia from different varieties of alfalfa also differ, he concludes that the two are identical. Tehon and Daniels (1925:717) following Elliott's (1917:471) suggestion that the fungus is not a good member of the genus Macrosporium, and assuming that the forms on clover and alfalfa are identical, erect a new genus, Thyrospora, with *T. sarcinaeforme* (Cav.) comb. nov. as the type species. The chief character of Thyrospora is the echinulate or warty conidia.

The investigations of the present writer seem to shed some light upon this nomenclatorial tangle. In connection with laboratory tests of fungicides more than 250,000 spores produced in pure cultures from red clover were examined with the low power of the microscope. Not a single spore with spiny walls was ever seen. Numerous supplementary observations of both stained and unstained spore walls with the high power of the microscope and with oil-immersion objectives have substantiated this conclusion. Spores from lesions on alfalfa leaves invariably appear obviously roughened. Examination of specimens of these fungi on clover and alfalfa from Illinois, which were sent to the writer by Dr. L. R. Tehon, revealed that the spores from the clover leaves are smooth, whereas spores from alfalfa leaves are spiny.

Furthermore, the leaf lesions on the two suscepts are different. Those on alfalfa contain much more yellow than those on red clover, although this may be a suscept response. Single conidial cultures from the two plants grown under identical conditions are distinct, as shown in figure 3. Cultures from red clover are velvety black, with practically no aerial mycelium and no signs whatsoever of perithecial formation. Smooth-walled conidia are produced in great profusion. Cultures from lesions on alfalfa leaves, on the other hand, are very much lighter in color, possess none of the velvety black character, have white fluffy aerial mycelium, with but little dark-colored submerged mycelium, and bear only a few conidia that are spiny. These cultures produce a limited number of black sclerotioid bodies which appear to be perithecial primordia. Incidently,



A

B

Figure 3. comparison of single spore culture of macrosporium sarcinaeforme isolated from leaves of (a) red clover and (b) alfalfa if these could be made to produce mature ascospores, they probably would be those of *Pleospora herbarum*, as described by Gentner (1918). This fungus has been reported from a large number of herbaceous stems. Ultra-violet irradiation, following the method used by Stevens (1928), however, has not induced the perithecia to mature. The writer has obtained single ascospore cultures of what he takes to be *P. herbarum* from overwintered red-clover stems two seasons in succession. These gave cultures identical in every respect with those of Macrosporium from lesions on alfalfa leaves, including the production of perithecial primordia and spiny conidia. Neither in morphological characters nor in cultural reactions does this fungus correspond to *M. sarcinaeforme* as isolated from leaf lesions on red clover. It is probable that the Macrosporium from alfalfa and its possible sexual stage, *P. herbarum*, occurs on alfalfa and red clover, but that *M. sarcinaeforme* from red clover is limited to that suscept in nature.

These observations indicate that Tehon and Daniels (1925) are not justified in including the two pathogenes in the same species, especially if the spiny character of the spore walls is constant. Elliott (1917:467) remarks that " echinulation occurred at all times in all the species studied. In some cases part of the spores from a colony were echinulate while others were smooth." The author has found no evidence of variability in the surface character of the conidia of *M. sarcinaeforme* from red clover. Inasmuch therefore as *M. sarcinaeforme* Cav. was not described with spiny spores, it cannot be incorporated as the type species nor even included in the new genus Thyrospora.

Since the original description undoubtedly refers to a smooth-spored fungus on the leaves of red clover, the name *Macrosporium sarcinaeforme* Cav. is reserved for that species. The purposes of this paper do not warrant a comprehensive discussion of the systematic position of the alfalfa organism aside from saying that it probably is not identical with the one on red clover.

Pathogenicity. Malkoff (1902:284) first attempted to show that *Macrosporium sarcinaeforme* Cav. was pathogenic by inoculating detached leaves in moist chambers with spores. Characteristic spots appeared in from five to seven days, but no reisolations are reported. Bain and Essary three years later (1905:503) say, "The Macrosporium disease appears capable of destroying the clover plant unassisted by any other parasite, though this statement is based only on inspection in the field." Krakover completed Koch's rules of proof for the organism. The fungus has been isolated a number of times by this author and has been grown in pure culture. It was then inoculated onto healthy red-clover plants. The typical spots were produced, and the fungus was reisolated, giving cultures indistinguishable from the original. Spores from these reisolations again produced the disease when inoculated onto other red-clover plants. There seems to be little doubt of the ability of the fungus to produce the disease.

Life history. The complete life history of the fungus has not been worked out. Evidently the sexual stage is unknown as yet.

Gentner (1918), Krakover (1917: 315), and Milburn (1915: 72) report the fungus in the seed. It would appear that the occurrence of the fungus within the seed argues for the possibility that primary cycles sometimes are initiated by rain splashing spores from the seed up on young foliage. Krakover (1917) placed soil from an infected field in pots in the greenhouse. Sterile seed when planted failed to produce infected plants as long as he watered the plants from below; but when he watered them from above with the hose, they immediately became infected, indicating that splashing rain is an important factor. Probably this is the chief inoculating agent, the spores coming either from the diseased seed or the infected soil that constitutes the inoculum.

The effect of some of the factors governing spore germination has been tested by the writer.

In studying the effect of temperature on germination, the spores almost invariably have been allowed to remain in each temperature chamber until all the spores germinable at that temperature grow. This gives a flat curve when the results are expressed graphically, but it indicates to what extent spores may be expected to germinate under given temperature conditions. Although it is desirable to learn the minimum time for maximum germination at each temperature, this has not always been done.

In one experiment conidia were germinated in temperature chambers ranging all the way from 3° to 35° C., with intervals of 6 degrees. Germination after forty-two hours was essentially equal in all the incubators, being from 96 to 99 per cent. The germination at 3° C. was 97.6 per cent and that at 35° C. was 99.2 per cent. Obviously the spores are capable of germinating over a very wide range of temperature.

TABLE 5. EFFECT OF TIME UPON CONIDIAL GERMINATION OF MACROSPORIUM SARCINAEFORME AT 3° AND 25° CENTIGRADE

3° Centigrade			25° Centigrade		
Time elapsed (hours)	Number of spores	Per cent of germination	Time elapsed (hours)	Number of spores	Per cent of germination
2	436	1.4	2	476	7.0
3	441	4.0	3	497	35.9
3¾	462	6.1	3¾	450	75.6
5	504	9.0	5	465	93.7
6	499	9.8	6	462	95.9
9	472	12.5	7	488	97.3
13	432	42.3	8	485	98.2
24	459	84.3			
26	458	90.6			
42	423	97.7			

Another experiment was laid out the following day to get a time curve for the germination at 3° and 25° C., since it had just been shown that germination was the same at both temperatures. The curve should show a more rapid rise at the higher temperature. That such did prove to be the case is borne out by the data in table 5 and by figure 4.

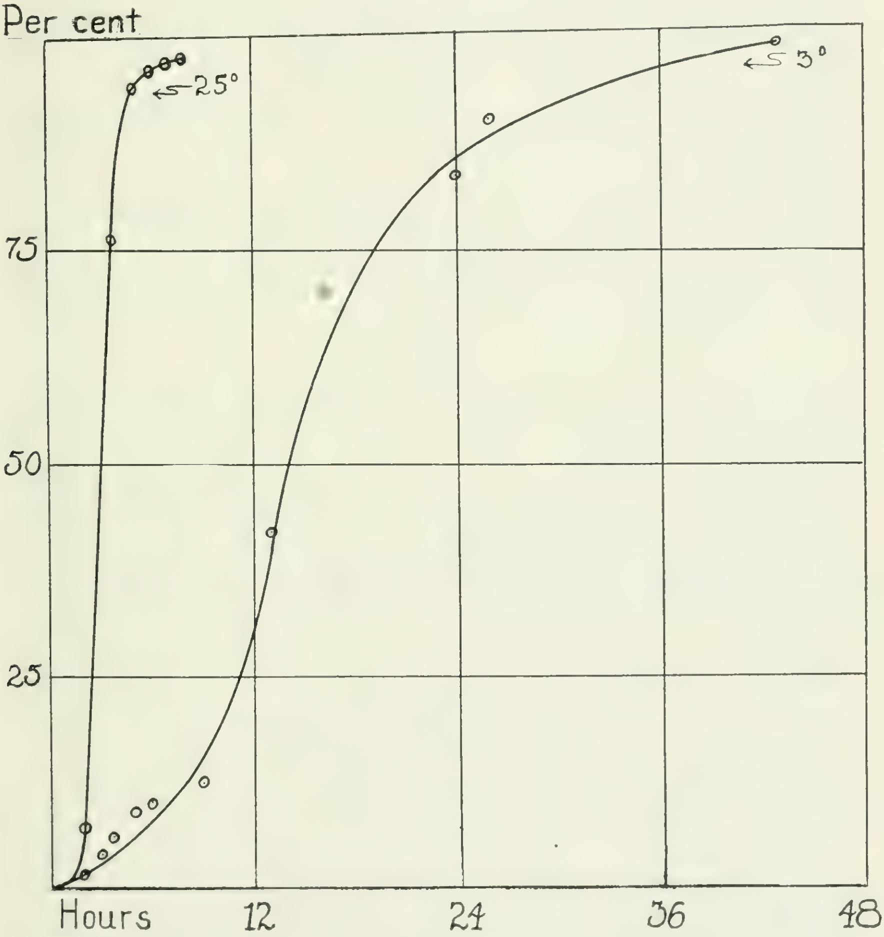


FIGURE 4. GERMINATION OF CONIDIA OF MACROSPORIUM SARCINAEFORME AT 3° AND 25° C., SHOWING LAG AT LOWER TEMPERATURE BUT GOOD FINAL GERMINATION

Krakover (1917:284) by examining the "infection drop" directly under the microscope found that penetration occurs directly through the cuticle after about fourteen hours. Although entrance through stomata occurs, it is uncommon.

At first it was thought that the concentric zones might be cicatrices laid down by the suscept in successive attempts to halt the invasion of the pathogene, but sections through the lesions show, however, neither cicatrice (Cunningham, 1928) nor thickenings of the cell walls which might hinder the advance of the hyphae into adjacent living tissues. In fact, no good explanation of the concentric zones can be offered at this time. The cells within the holonecrotic area are collapsed and browned with disintegrated chloroplasts.

One may assume from the work of Krakover in which he inoculated plants from infested soil that the fungus was living saprophytically in that soil. The vigorous growth of the fungus in artificial culture indicates that it is not unamenable to a saprophytic existence.

Secondary cycles occur throughout the season, and are chiefly responsible for the distinctive development of the disease.

Control

Milburn (1915:72) upon finding the fungus in the seed recommends seed treatment. To what extent this may be effective and practicable remains to be determined.

Because of the absence of the disease from the experimental areas, two-seasons field experiments with sulfur dust have given no indications of value. On the other hand, a large amount of data regarding the effect of sulfur and copper dusts upon the germination of conidia on glass slides has been accumulated in the laboratory.[2] The effect of the fungicides in inhibiting infection in the greenhouse has been studied as well, but to a less extent.

Laboratory experiments

Effect of sulfur. The results of several experiments on the toxicity of sulfur to spores of *M. sarcinaeforme* are presented in table 6.

This table shows that elemental sulfur in these forms is almost, if not utterly, incapable of inhibiting germination of the spores. In order to see if soluble sulfur, on the other hand, would inhibit germination, a test

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[2] The technic for the laboratory testing of fungicides reported in this paper is essentially that long used in the Department of Plant Pathology at Cornell University, and described recently by McCallan (1930).

The writer's methods have differed from those of McCallan in a few minor details. Duplicate slides for each treatment were used in each experiment. Each slide contained four separated drops of a spore suspension diluted approximately to 50 spores to each drop, as examined by the low power of the microscope. In determining the percentage of germination, all germinated and ungerminated spores were counted separately in one field of the low power of the microscope. Invariably the materials were dusted on dry slides on which the spore suspensions were placed subsequently. Bank's colloidal sulfur dust is a patented product prepared by fusing finely ground sulfur with bentonite, a colloidal clay, and regrinding. Kolodust is a trade name applied to a dilution of Bank's colloidal sulfur in 300-mesh dusting sulfur.

TABLE 6. EFFECT OF ELEMENTAL SULFUR ON CONIDIAL GERMINATION OF MACROSPORIUM SARCINAEFORME

Experiment no.	Check		Bank's colloidal sulfur dust		Kolodust		200-mesh dusting sulfur	
	Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination	Numner of spores	Per cent of germination
1	442	99.3	394	93.4	.....	.....	416	97.4
2	411	98.1	409	97.4	397	98.5	412	95.8
3	436	99.6	417	99.3	408	99.5	443	98.8
4	469	100.0	427	97.0	450	99.2	439	99.3
Total or average	1,758	99.2	1,647	96.8	1,255	99.0	1,714	97.8

with N/10 potassium sulfide in comparison with a check and Kolodust was made. The results of this test are given in table 7.

TABLE 7. EFFECT OF SOLUBLE SULFUR ON CONIDIAL GERMINATION OF MACROSPORIUM SARCINAEFORME

Check		Treatments			
		Kolodust		N/10 potassium sulfide	
Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination
211	99.5	212	99.5	201	88.6
225	99.6	196	99.5	170	87.0

The table shows that soluble sulfur reduces the germination about 10 per cent. It appears then that the complete nontoxicity of elemental sulfur shown in table 6 is due in part to its insolubility in the presence of germinating spores rather than to the tolerance of the protoplasm to the sulfur ions exhibited in table 7.

Effect of copper. A large number of tests were performed with copper dusts during the course of the experiments. In table 8 only the results

TABLE 8. EFFECT OF 0.5-PER-CENT COPPER-LIME DUST ON CONIDIAL GERMINATION OF MACROSPORIUM SARCINAEFORME

Experiment no.	Check		Dust	
	Number of spores	Per cent of germination	Number of spores	Per cent of germination
1	419	95.5	376	93.4
2	446	98.5	408	0.3
3	417	98.9	441	58.1
4	487	98.7	442	95.3
5	456	97.1	423	68.5
6	442	97.5	356	48.8
7	417	98.1	465	9.7
Average or total	3,084	97.7	2,911	53.3

with the standard monohydrated copper-lime dust are included. Since in all the tests the 6.8-per-cent metallic copper, diluted with lime, talc, or bentonite, prevented germination entirely until a content of 0.5-per-cent metallic copper was reached, the data in the table refer only to the latter dilution.

The fluctuations of the determinations around the mean of 53.3 indicates that the 0.5-per-cent copper content is at the lower toxic limit.[3] Many factors probably operate to vary the germination from day to day, but the chief one is the variation in the amount of copper on the slides. These tests indicate that a copper dust containing 1 per cent or more of metallic copper should prove effective in field work.

A study was made on the effect of diluents upon the efficiency of the copper lime. In making these tests, one of three substances, lime, talc, or bentonite, was generally used as a diluent for the copper-lime dust. The results obtained from testing these alone for their fungicidal properties are presented in table 9.

The probable error of the differences reveals that the lime and the talc appear slightly toxic, but the differences are so slight that they probably do not have any great significance. Bentonite appears not to be toxic at all.

Greenhouse experiments

A preliminary experiment made in the greenhouse when plants were inoculated after having been sulfured, presaged the results just set forth even before any spores were ever germinated on slides in the presence of fungicides. Plants of mammoth red and medium red clover in pots were selected for inoculation and were divided into two lots, one of which was sulfured with Bank's colloidal sulfur dust and the other was left untreated as a check. The plants were placed in a large moist chamber

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[3] One experiment with *Macrosporium sarcinaeforme* demonstrated that a dust diluted to a point near what appears to be the minimum of toxicity inhibits germination in varying degrees, depending upon the amount deposited on each slide. Copper-lime dust diluted to 0.5 per cent with talc was applied in heavy and light dosages, as is shown in the following table where the results of the germination tests appear. Except for the check, which was prepared only in duplicate, each treatment was replicated four times each in duplicate, making actually ten tests in all, so that the results should be significant.

EFFECT OF COPPER DOSAGE ON CONIDIAL GERMINATION OF MACROSPORIUM SARCINAEFORME

Treatment	Number of spores	Per cent of germination
Check	487	98.8
Heavy dose	2,161	42.1
Light dose	2,100	98.2

A large number of tests with the same fungus and 1-per-cent copper-lime dust diluted in talc gave no germination, regardless of the thickness of the application. Hence it appears that 0.5 per cent is at or near the minimum point of toxicity. Variations in dosage may be overcome by replication of the experiments.

TABLE 9. Effect of Diluents on Conidial Germination of Macrosporium Sarcinaeforme

Experiment no.	Per cent of germination			
	Check	Lime	Talc	Bentonite
1	98.5	92.1	91.0	....
2	96.0	94.7	67.8	....
3	99.5	96.6	94.5	98.5
4	99.1	96.1	97.6	98.9
5	96.1	94.2	98.6	97.7
6	94.8	97.6	95.2	97.7
7	98.6	81.3	89.0	97.8
8	97.0	86.9	93.5	95.4
9	98.7	97.5	98.6	98.5
10	98.2	99.0	99.1	99.0
11	99.5	97.7	99.5	99.5
12	98.2	98.5	96.9	98.5
13	98.8	98.6	99.1	....
14	98.7	98.6	....	....

Error of the mean*: check, 98.0 ± 0.02; lime, 94.9 ± 0.91; talc, 95 ± 0.07 and bentonite, 98.1 ± 0.01.
Error of the difference: check and lime, 3.05 ± 0.91; check and talc, 2.60 ± 0.07; check and bentonite, 0.14 ± 0.02.

* The probable error of the mean in this bulletin has been calculated according to Bessel's formula:

$$E_m = 0.6745 \sqrt{\frac{\frac{E\ (v-g)^2}{n} - C^2}{n \text{ or } n-1}}$$

for twenty-four hours, and then were transferred to a greenhouse bench until data were taken. The results of two experiments, one conducted in the spring and one in the fall, are given in table 10.

TABLE 10. Effect of Sulfur on Infection by Macrosporium Sarcinaeforme in the Greenhouse

Experiment no.	Pot no.	Check			Sulfured		
		Number of healthy leaves	Number of diseased leaves	Per cent of healthy leaves	Number of healthy leaves	Number of diseased leaves	Per cent of healthy leaves
1	1	188	220	53.9	294	186	38.7
	2	255	50	16.3	205	66	24.4
	3	136	64	32.0	161	48	22.9
	4	119	58	32.7	110	58	34.5
	5	154	160	50.9	187	80	45.2
Average...	...	...	...	39.3	...	...	31.4
2 *	1	118	178	39.9	275	221	55.4
	2	103	103	50.0	198	169	53.9
	3	210	152	58.0	96	51	65.3
	4	51	55	48.1	112	145	43.6
	5	79	32	71.1	50	45	52.6
	6	114	112	50.4	108	64	62.8
	7	...	...	....	26	22	54.1
	8	...	...	....	81	45	64.3
	9	...	...	....	16	62	20.5
Average...	...	...	...	45.3 ± 1.86	...	...	42.9 ± 2.4

* E_d in experiment no. 2 = 2.4 ± 2.62.

It is seen from the table that on the basis of the percentage of leaves infected, a control of 8 per cent was obtained in experiment no. 1, but the difference is probably not even out of the range of experimental error. The difference due to treatment is much less in experiment no. 2 than in experiment no. 1 and is not significant.

It is evident from table 10 that the experiments in the greenhouse confirm the laboratory tests, showing that *Macrosporium sarcinaeforme* is tolerant of sulfur.

A single test was made in the greenhouse to see if copper-lime dust is as effective in preventing infection as it had been shown to be in inhibiting spore germination. Standard copper-lime dust was applied before inoculation to the leaves that had been moistened previously with tap water. The results of the counts, which were made after eight days had elapsed, appear in table 11.

TABLE 11. EFFECT OF COPPER-LIME DUST ON INFECTION BY MACROSPORIUM SARCINAEFORME IN THE GREENHOUSE

Treatment	Pot no.	Number of leaves	Number of spots	Number of spots per leaf
Undusted checks	1	184	1,065	8.07
	2	230	1,372	8.02
	3	155	631	6.37
	4	178	737	5.94
Average	...	...	....	7.10 ± 0.37
Dusted	5	150	407	4.42
	6	252	507	3.17
	7	229	483	3.63
	8	126	239	3.09
Average	...	...	....	3.58 ± 0.202

Difference = 3.52 ± 0.42

$\frac{D}{E_d} = 8.38$

Obviously the copper did prevent some infection, because the leaves in the dusted lot averaged only about 50 per cent as much infection as the undusted leaves. The laboratory tests, however, indicated that better control should have been obtained. A single greenhouse experiment is insufficient to warrant the conclusion that copper-lime dust would prove ineffective under field conditions, especially in view of the results obtained in the laboratory.

When all the tests are considered, it appears that sulfur dusts or sprays cannot be relied upon to give control of this disease but that copper-lime dusts are promising.

CLOVER RUSTS

All the rusts of the common clovers are included under the heading *Clover rusts* because of their close relationship. This has been deemed necessary since much confusion in their nomenclature exists through failure to distinguish between the fungi on the various clovers. Emphasis is placed on the rust of red clover, and the points of difference between it and the others are emphasized where necessary.

Suscepts

Arthur (1912:255) regards the rust on red clover as identical with that on crimson clover and on zigzag clover (*Trifolium medium*). He believes also that the rusts on alsike and white clover are identical but are different from that on red clover. The fungus on alsike and white clover also attacks the following plants, according to Arthur: *Trifolium amabile* H. B. K., *Trifolium amphianthum* T. & G., *Trifolium incarnatum* L., and *Trifolium logani* House. He believes that crimson clover harbors both rusts. Davis (1924 a), however, has separated the alsike rust as a distinct disease.

A perusal of the literature reveals only two references to varietal susceptibility. A meager report by Mains from Indiana states that Italian red clover is resistant (Stakman, 1922:243). An anonymous contributor (1923) from the same State says that "two hundred seven selections were made from a large number of plants studied which showed a high resistance or immunity to rust, many of these being resistant to mildew."

The disease

Writers, almost without exception, have referred to these diseases as *clover rust*. Freeman (1905:292) speaks of them as *clover leaf rust*, but since many other parts of the plants besides leaves are attacked, this name is hardly satisfactory. Since each of the three pathogenes are definitely restricted to certain clovers (Davis, 1917), it seems best to combine the common name of the suscept with the term *rust*, to distinguish the three diseases. They therefore are called *red-clover rust*, *alsike rust*, and *white-clover rust*, respectively.

History and range

Red clover probably is rusted wherever it is grown, for it has been reported from 33 States, from 5 Canadian provinces, from 17 countries of Europe, from Asia (Sydow and Sydow, 1910:131-134), and from Australia (McAlpine, 1906:42-44). Although the rusts of alsike and white clover have not been reported as frequently as has that of red clover, they apparently are as nearly ubiquitous.

Economic importance

Usually the rusts of clover have been regarded as of little importance. Most of the records in the *Plant Disease Reporter* read like this, " common but for the most part of only slight importance " (Haskell, 1919:156). It is interesting that, on the contrary, the early references to the disease in America would seem to indicate that it was worse in years gone by than it is now. It is " of great importance " wrote Freeman in 1905 (1905:166). Miss Howell (1890 a:129) stated that it " prevailed to such an extent during the several wet, cool seasons preceding 1890 . . . that it must be regarded as a disease likely to affect seriously, under conditions favorable to its development, an agricultural crop." Pammel (1891:51) in Iowa said that some plants were so severely attacked that the hands became reddened on touching them. Underwood (1888:302) two years earlier estimated that from 5 to 20 per cent of the value of the clover was being destroyed in the vicinity of Syracuse, New York.

According to Lobik (1922), on the other hand, the disease stimulates the plants attacked, so that a heavier production of both green parts and seed results. Such a statement requires more experimental demonstration.

With the exception of that on alsike, the diseases do not now seem to be of much economic importance in New York. Rust can be observed in practically every planting of red clover after midsummer and occasionally as early as June 15, but in no case has severe infection been observed before the first, and most important, cutting was removed. White-clover rust does appear to be destructive to the plants in many lawns and waste places.

Symptomatology

Although the writer has not yet seen the aecial stage on red clover, Hoffer (1917:326) remarks that " the striking feature of the leaves bearing aecia is that they blacken and wilt in a very short time." He believes that for this reason the aecial stage is commonly overlooked. The aecial lesions, which are hyperplastic on white and alsike clovers, kill the affected leaves only after a considerable time. The pycnia appear first as honey-yellow dots on a much swollen lesion on vein, petiole, or stem of the plant. Aecia, which soon appear, are of about the same color. The swellings are often profound, causing the petiole, for example, to swell to several times normal size. It may also bend at this point. When veins are attacked, the leaf blade becomes greatly distorted.

The uredinia are practically alike on all three clovers. They occur, as shown in figure 5, as minute pale cinnamon-brown pustules on any exposed part of the plant, calyces, stipules, stems, petioles, and leaves. They occur on leaves first on the lower surface, and then on the upper. When uredinia are scattered widely over the blade, little or no yellowing

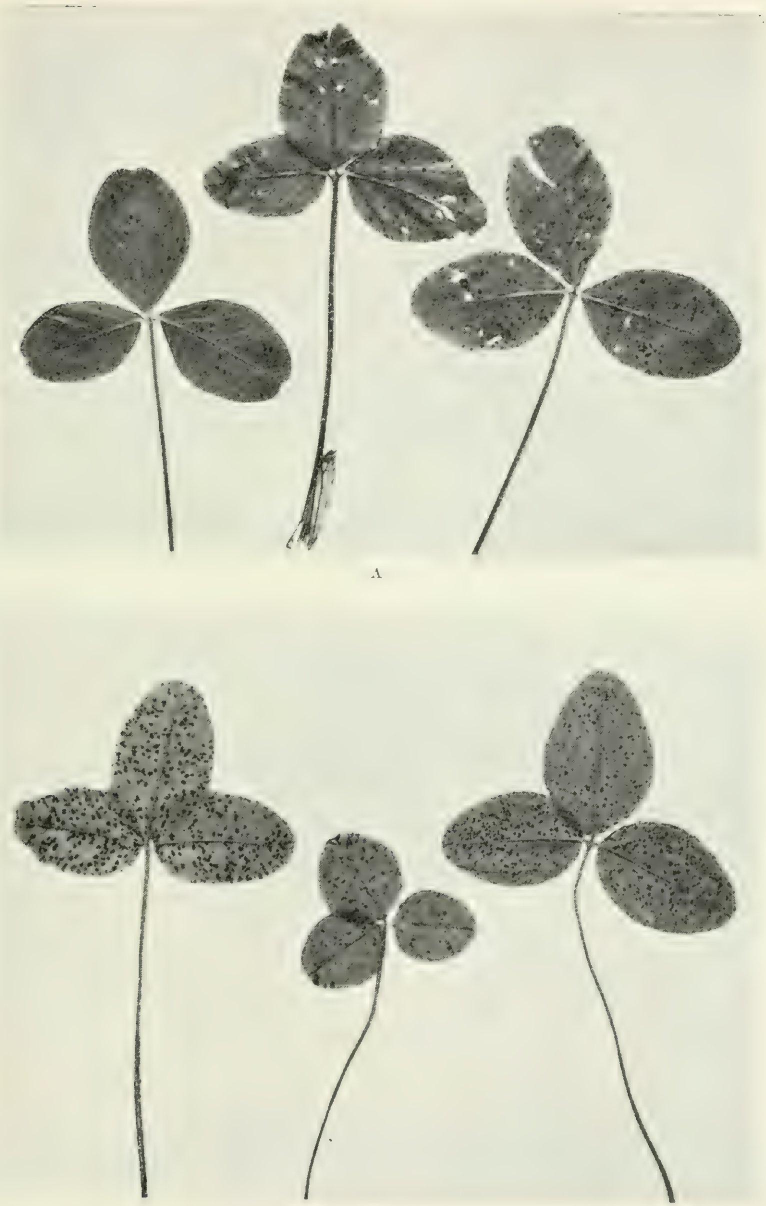

Figure 5. UREDINIAL SORI OF UROMYCES TRIFOLII FALLENS ON LEAVES OF RED CLOVER (A) AND OF UROMYCES TRIFOLII HYBRIDI ON LEAVES OF ALSIKE CLOVER (B)

or other suscept response occurs. If they be numerous, however, the leaf sooner or later succumbs, first turning yellow around the spots. Soon the entire blade is involved and finally turns brown and then falls off. The uredinia do not cause evident hypertrophy.

The telia are distinguishable from the uredinia only by their slightly darker brown color

Etiology

Name, history, and classification of the pathogenes. The nomenclature of the various rust fungi on clovers has been so muddled that it is untangled with difficulty. Kern (1911:3-6) gives a good history of the situation up to 1911. He referred the rust on red clover to *Uromyces fallens* (Desm.) Kern, and this disposition seems to be a good one. He was puzzled about the report of aecia by Miss Howell (1890:131) and believed that they arose as a contamination of the white-clover rust. He listed the aecial and pycnial stages as lacking and suggested the possibility that they occurred on Euphorbia. Since the publication of Kern's paper, the aecial stage has been reported independently by Hoffer (1917:325-326), by Davis and Johnson (1917:75), and by Melhus and Diehl (1917:70), proving this rust to be autoecious as are the other clover rusts.

Prior to the paper of Kern, American plant pathologists placed all the clover rusts in one or another species of Uromyces, but referred them chiefly to *Uromyces trifolii*; some ascribing the specific name to Albertini and Schweinitz, some to Winter, and some to Hedwig.

Liro (1906) already in 1906 had pointed out correctly that the red-clover rust should be differentiated from the rusts on white and alsike clover. He placed the forms on alsike and white clover in *Uromyces trifolii-repentis* (Cast.) Liro, retaining that on red clover in *Uromyces trifolii* (Hedw. f.) Lev.

With *Uromyces fallens* as the name for the rust on red clover and *Uromyces trifolii-repentis* for the one on white and alsike clover, the nomenclature of the clover rusts rested until 1924 when Davis (1924 a: 203-219) established a new species, *Uromyces hybridi*, of the alsike form on the basis of suscept specificity of the three fungi and on small differences in the aecial and pycnial stages which he found. Arthur (1929:350-351) in his new book on the plant rusts considers the common clover rusts all as *Uromyces trifolii*, consisting of physiologic forms *U. trifolii fallens* on red clover, *U. trifolii hybridi* on alsike clover, and *U. trifolii trifolii-repentis* on white clover.

Pathogenicity. Miss Howell (1890 b:228) by inoculation experiments first formally connected the various spore forms on the clovers. She procured aecia from telial inoculations and uredinia from aecial inoculations. Telia developed later. Liro (1906:11–15) also proved the pathogenicity of these rust fungi by inoculation.

Life history. The general features of the life history of the clover-rust fungi appear to be those of similar autoecious long-cycled rusts. The telia overwinter on debris of diseased plants, where they produce the sporidia in the spring that infect the young leaves and produce aecia. No detailed studies appear to have been made of the phenomena of inoculation, incubation, and infection in the primary cycle in any of the clover rusts.

Aecia of the primary cycle furnish the inoculum for the first secondary cycles, and uredinia serve in the same capacity for the subsequent secondary cycles. Both aeciospores and urediniospores are borne by the wind to new infection courts.

For germination studies with the rust spores, leaves bearing fresh sori were collected in the field. The spores were washed from these leaves into Syracuse watch glasses with a stream of aerated distilled water from a wash bottle, as suggested by Doran (1922), in order to get mature spores and to avoid injuring them. The data on white-clover rust are very inconclusive because of the difficulty in obtaining good germination of the spores used as checks. It is assumed that no valid conclusions can be drawn when the percentage of germination of the checks is less than 90 per cent.

To study the rôle of temperature in germination, duplicate slides of urediniospores were placed at various temperatures in the incubators. The data presented in table 12 are based on an average of several such experiments.

TABLE 12. EFFECT OF TEMPERATURE ON UREDINIOSPORE GERMINATION OF UROMYCES TRIFOLII HYBRIDI AND UROMYCES TRIFOLII FALLENS

Temperature (degrees C.)	*Uromyces trifolii hybridi*			*Uromyces trifolii fallens*		
	Number of tests	Number of spores	Per cent of germination	Number of tests	Number of spores	Per cent of germination
3	4	825	41.8	4	812	89.2
9	4	893	87.4	5	1,053	94.9
12	1	172	91.2	....	....	....
15	4	838	91.5	4	736	95.7
18	3	667	93.8	....	....	....
21	4	1,159	92.6	2	315	97.4
25	5	2,104	94.8	4	885	93.2
33	5	2,288	15.9	4	865	6.0

It appears that 3° C. is somewhat too low for good germination for both fungi, and that 33° C. is entirely too high. There is, nevertheless, a wide range of temperatures within which these spores readily germinate. Little is known of infection phenomena. Waters (1928:189) found that the first uredinia appear after about eight or nine days on leaves of alsike clover inoculated in petri dishes. Telia begin to form in nature about October 1. Waters (1928:189) has shown recently that telia may be induced experimentally by three treatments: starvation of the suscept, with the later addition of a rich food supply; sudden change of the suscept from a well-fed condition to a stage of starvation; and continued supply of food, with a gradual dying of the suscept cells and a lowering of metabolism. The latter condition probably exists in nature, for telia of the red- and alsike-clover form have not been seen before the latter part of September or the first of October. Sydow and Sydow (1910:132) state, however, that telia may appear on white clover from May onward. Teliospores are not greatly different morphologically from urediniospores except that they are darker in color and have a slightly thicker wall. They have also one germ pore, while the urediniospores have several arranged equatorially. Teliospores may be borne directly in the same sorus with the urediniospores or in separate sori. The teliospores serve to carry the fungus over the winter.

Epiphytology

Miss Howell (1890 a:129) maintains that the clover rusts are most prevalent in cool, wet seasons. If this were true, the fungus should be at its height in the spring rather than in dry midsummer or after as the writer has always found it.

Control

No actual experimental work on the control of clover rusts seems to have been reported in the literature.

Miss Howell (1890 a:139) advocates plowing under the second crop and burning over diseased fields in the autumn. These opinions found subsequent expression in the writings of Pammel (1891:55) and of Freeman (1905:292). Miss Howell (1890 a:139) said that "the application of fungicides seems impracticable."

Until the work of Kightlinger (1925) most workers inclined to the view that rusts could not be controlled with fungicides. Much recent work has shown (Greaney, 1928) that wheat rusts may be controlled with sulfur dust. The work herein reported appears to be the first dealing with the control of clover rusts with fungicides.

Effect of sulfur. The results obtained with various sulfurs are extensive and seem to be rather conclusive. Data on the white-clover rust are not presented since the spores germinate poorly. The data on the red-clover and alsike-clover forms are given in tables 13 and 14.

TABLE 13. EFFECT OF SULFUR ON UREDINIOSPORE GERMINATION OF UROMYCES TRIFOLII HYBRIDI

Experiment no.	Check		Bank's colloidal sulfur dust		Kolodust		Undiluted 200-mesh sulfur	
	Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination
1	873	96.9	867	78.4	....	....	749	88.5
2	923	92.6	....	....	871	60.7	928	88.9
3	417	97.5	489	27.8	408	42.4	....	....
4	429	95.8	567	19.4	537	39.1	....	....
5	435	98.8	418	12.5	448	21.0	460	43.0
Total or average	3,077	96.3	2,341	34.5	2,264	40.8	2,137	73.5

TABLE 14. EFFECT OF SULFUR ON UREDINIOSPORE GERMINATION OF UROMYCES TRIFOLII FALLENS

Experiment no.	Check		Bank's colloidal sulfur dust		Kolodust		Undiluted 200-mesh sulfur	
	Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination	Number of spores	Per cent of germination
1	797	88.1	1,115	3.8	....	....	1,100	84.5
2	1,000	92.0	1,023	24.6	....	....	1,221	69.9
3	627	91.6	645	56.9	488	75.8	652	74.9
4	413	97.7	371	30.2	330	33.2	366	75.2
5	473	99.5	400	0.0	....	....	....	....
6	453	96.2	492	2.8	439	22.3	....	....
7	515	94.2	495	7.8	445	17.5	462	26.7
8	447	98.2	519	7.0	466	19.7	....	....
Total or average	4,725	94.7	5,060	19.0	2,168	33.7	3,801	66.2

The effects of the various sulfurs on the spores are illustrated in figure 6. These drawings, in conjunction with the rather low germinations obtained, indicate that sulfur offers promise for field work in controlling the clover rusts. The reduction in germination caused by any of the dusts is far from complete, yet the tubes are so short and abnormal that many of them would probably never get into the stomates. Another interesting fact brought out by tables 13 and 14 is that the sulfurs reduced the germination more in 1928 (experiments 5 to 8) than in 1927 (experiments 2 to 4). This may be due to the expression of the influence of seasonal differences on the sensitiveness of the urediniospores.

Effect of copper. Copper-lime dust was not tested against the germination of the urediniospores except in 1928. It would be interesting to have data from 1927 to see if the sensitiveness of the spores to this fungicide

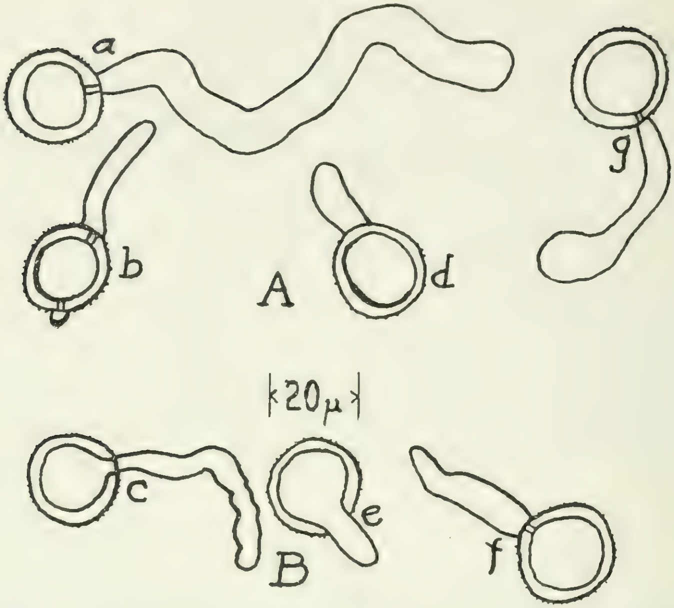

FIGURE 6. GERMINATING UREDINIOSPORES OF CLOVER-RUST FUNGI, UROMYCES TRIFOLII HYBRIDI (A) AND UROMYCES TRIFOLII FALLENS (B), SHOWING THE TOXIC EFFECTS OF SULFUR AND COPPER FUNGICIDES

a, Check; *b* and *c*, in copper-lime dust; *d*, *e*, and *f*, in Bank's colloidal sulfur dust; *g*, in Kolodust

was less in 1927 than in 1928, as was the case with sulfur. The data on this fungicide for both rusts are given in table 15.

TABLE 15. EFFECT OF COPPER-LIME DUST ON UREDINIOSPORE GERMINATION OF UROMYCES TRIFOLII HYBRIDI AND UROMYCES TRIFOLII FALLENS

Uromyces trifolii hybridi					*Uromyces trifolii fallens*				
Experiment no.	Check		7.2-per-cent copper-lime		Experiment no.	Check		7.2-per-cent copper-lime	
	Number of spores	Per cent of germination	Number of spores	Per cent of germination		Number of spores	Per cent of germination	Number of spores	Per cent of germination
1.......	417	97.5	416	33.9	1	473	99.5	439	15.2
2.......	429	95.8	438	35.1	2	453	96.2	411	22.5
3.......	435	98.8	438	33.3	3	515	94.3	447	46.6
	....	....	....	....	4	447	98.2	469	34.4
Total or average	1,281	97.4	1,292	34.1		1,888	97.0	1,766	29.7

The effect of the copper on the germination is illustrated also in figure 6. A comparison of this figure and the data presented in table 15 with the data in tables 13 and 14 on the effect of sulfur shows that sulfur is more likely to control the disease in the field than is the copper-lime dust.

Effect of lime. A few tests were conducted to see what effect lime alone would have in reducing the germination of the urediniospores. The results are presented in table 16.

TABLE 16. EFFECT OF LIME ON UREDINIOSPORE GERMINATION OF UROMYCES TRIFOLII HYBRIDI AND UROMYCES TRIFOLII FALLENS

Experiment no.	*Uromyces trifolii hybridi*			*Uromyces trifolii fallens*		
	Number of tests	Number of spores	Per cent of germination	Number of tests	Number of spores	Per cent of germination
1	2	452	81.9	2	455	97.0
2	2	431	90.8	....	....	....

When these results are compared with the checks, given in table 15, it appears that lime is not toxic to *Uromyces trifolii fallens* and only slightly so, if at all, to *Uromyces trifolii hybridi.*

PSEUDOPEZIZA LEAF SPOT

Suscepts

Many of the species of Trifolium have been listed by Jones (1919:5) as suscepts of Pseudopeziza leaf spot. The writer has collected good material on *Trifolium pratense*, *T. hybridum*, *T. repens*, and *T. medium*, and the first three species have been inoculated successfully with a pure culture isolated from *T. pratense.* Inoculations upon *Melilotus alba*, *Medicago sativa*, and *Vicia villosa* with the same culture were unsuccessful.

Williams (1927) reports that early varieties of red clover suffer more from this disease than do the later-maturing varieties. The less-susceptible ones are Vale of Clwyd (medium-early strain), American medium, and Bohemian. Italian, French, Swiss, and Silesian varieties he found to be the most susceptible.

The disease

This disease is usually referred to merely as *leaf spot.* Since it should be distinguished in some manner from the other leaf spots, the name *Pseudopeziza leaf spot* is used here.

History and range

According to the supplements of the *Plant Disease Reporter* this malady occurs from Vermont to California, and from New Brunswick, Canada, south to Virginia and Tennessee. It is found in practically every clover field in New York. In Europe, it occurs in Italy (Bivona-Bernardi, 1816), Wales (Stapledon and others, 1922:16), Russia (Jaczewski, 1916), Belgium (Lambotte, 1880:264), England (Massee, 1914), and Germany (Voges, 1909).

Economic importance

Pseudopeziza leaf spot undoubtedly is the most serious leaf disease of red clover in New York, as a reference to the survey data will show. From these data it appears that statements to the effect that no especial damage results (Haskell, 1924:307) are misleading. If New York be considered typical of the northern clover-growing States, then the disease is of great importance. Miss Massee (1914:65) states that the disease is far more prevalent and injurious in England than is generally suspected.

Symptomatology

This disease is easy to recognize in the field. The diagnostic symptom is the fimbriately margined, dark reddish, purple, dark olive, or even almost black spots which show on both leaf surfaces. The fimbriate margin does not occur invariably, but when it does, it is an unmistakable symptom of the disease. Such spots are shown in figure 7, A. Sometimes, especially late in summer, the fimbriate condition is almost wholly lacking. This seems to be due to the dry conditions under which the spots develop. Such spots are illustrated in figure 7, B. The fimbriate spot is most pronounced on *T. pratense* and *T. medium*, but it is usually apparent on *T. hybridum*. This character is much less prominent on *T. repens*. Furthermore, the general color tone of the spots on *T. hybridum* and *T. repens* is lighter than on *T. pratense* or *T. medium*.

In the greenhouse very young infections are much lighter in color than are the older spots observed in the field, matching Prout's brown or mummy brown of Ridgway's color standards (1912). It is practically impossible to diagnose this disease in its early stages in the field because the color of the lesions is not markedly different from that of the other leaf spots. Young spots resulting from artificial inoculation are illustrated in figure 7. The spots may vary from mere specks four or five days after inoculation up to 2 or 3 millimeters in diameter under the wet conditions that often prevail in thick stands of clover in the spring. The disease also appears as small elongated dark streaks on leaf petioles. Pure cultures of the pathogene have been obtained from such lesions.

Cunningham (1928:742) says that the histologic symptoms of this disease on red clover are the same as for those on alfalfa attacked by the congeneric parasite *P. medicaginis*.

> In the holonecrotic area [that is, the dead area in the center of the spot] the palisade cells are almost entirely replaced by the stroma of the fruit body while the cells of the spongy parenchyma are either collapsed or filled with the hyphae. In the plesionecrotic zone [that is, the diseased tissue surrounding the dead area] the epidermal cells may be collapsed. The

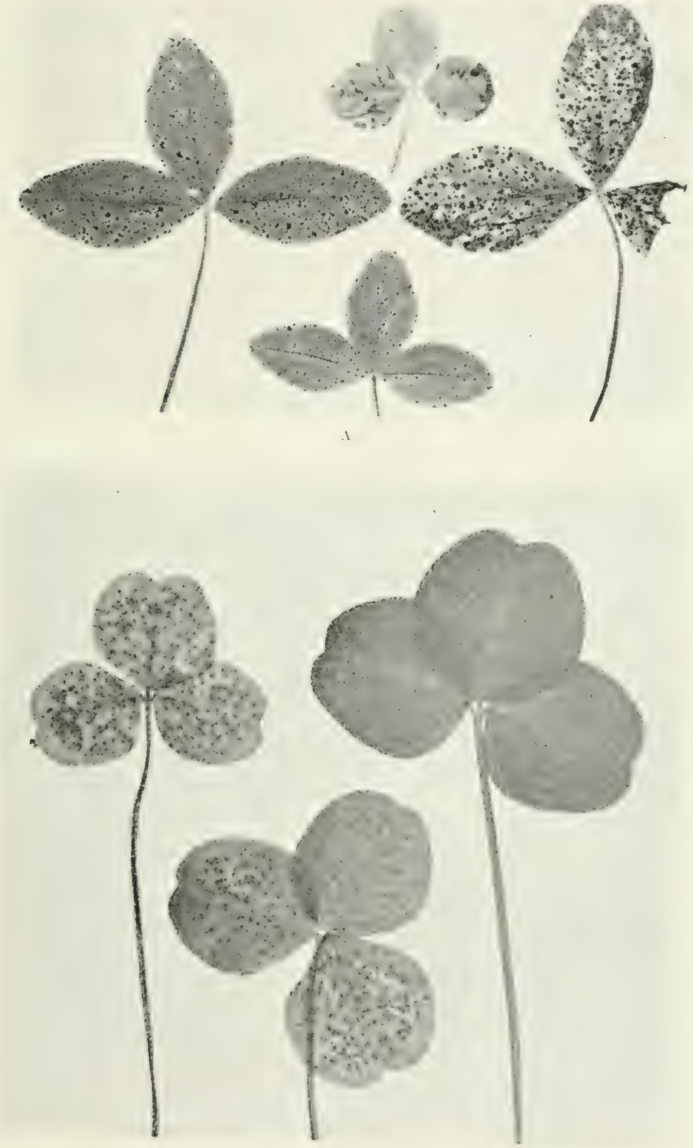

FIGURE 7. LESIONS CAUSED BY PSEUDOPEZIZA TRIFOLII ON LEAVES OF RED (A) AND ALSIKE (B) CLOVERS

The lesions on the alsike leaves are the result of artificial inoculation hyphae can frequently be seen in one or two cells beyond this point and occasional threads of hyphae can be seen in the mesophyll cells of this region and may extend to within two or three cells of normal tissue. The cells in this zone have their contents more or less disorganized and an occasional palisade cell is filled with some dense brownish substance, but there is little if any collapsing of cell walls. There is no evidence of cicatrice formation.

The minute apothecium in the center of older spots is another infallible evidence of the disease. After a rain or in moist weather these little fruit bodies push through the epidermis and glisten as minute amber-colored, jelly-like globules, but in drying they shrink and darken so that they are difficult to detect. It is these which make determination simple on the alsike and white clovers.

Etiology

Name, history, and classification of the pathogene. Pseudopeziza trifolii (Fr.) Fckl. is the cause of this leaf spot. The organism was described first in 1816 by Bivona-Bernardi as *Ascobolus trifolii* and was cited six years later by both Persoon (1822:342) and Fries (1822:165) under that name. In 1834 Madame Libert called it *Peziza trifoliorum* (1834:324). Boudier changed the name again in 1869 to *Phacidium trifolii* when he revised the Ascobolaceae (1869), but in the same year Fuckel (1869: 290) erected the genus Pseudopeziza, making *P. trifolii* (Biv.-Bernh.) Fckl. the type species. It has remained *P. trifolii* to this day, but the specific name is ascribed to various persons. Frequently, (Biv.-Bernh.) Fckl. is cited. The *h* in the second name is an error apparently first made by Fries (1822:165) and copied by subsequent writers. Persoon (1822: 342–343) omits the *h*. This authority is correct according to the so-called American code which accepts the very first specific name applied. If, however, the International code be followed (Briquet, 1912), the authorities are (Fr.) and Fckl. Oudemans (1921:875) lists the following as synonyms: *Ascobolus trifolii* Biv.-Bern., *Mollisia trifolii* Phill., *Peziza trifoliorum* Lib., *Phacidium trifolii* Lib., *Phacidium trifolii* Boud., and *Trochila trifolii* DeNot.

Much confusion existed for a time through the supposed identity of the forms on clover and alfalfa, but Jones (1919) has shown rather clearly that they are different. In this paper, therefore, the organisms on species of Trifolium are considered to be *P. trifolii* and those on species of Medicago to be *P. Medicaginis*.

Pathogenicity. Jones (1919) was the first person to grow *P. trifolii* in pure culture. The writer has isolated the fungus from red clover by planting pieces of leaves bearing very young lesions in separate tubes of oat agar. Apothecia are formed within a month if a bit of sporulating culture is placed at the top of an oat-agar slant and is allowed to discharge spores over it as pointed out by Jones (1919). Since the fungus grows very slowly at first, it is isolated only with difficulty. Subcultures grow somewhat more rapidly, however; but unless transfers are made frequently, the fungus tends to cease sporulating. By allowing a pure culture to discharge spores onto plants under a bell jar copious infections have resulted repeatedly on red and alsike clovers, and once a few minute spots showed on white clover. Reisolations were successful from the red and alsike clovers, giving a fungus indistinguishable from the original culture. No reisolation was attempted from the white clover. The results of these inoculation studies agree with those of Jones (1919:22), who inoculated red and alsike clover successfully but who did not use white clover.

Frequently infections are so plentiful on red and alsike clover that the leaves appear peppered with small brown spots, as shown in figure 7. Under such conditions apothecia are not developed.

Life history. Overwintered living leaves in the spring bear apothecia that discharge their ascospores to initiate the primary infections. Apothecia have been known to shoot their spores at least 5 millimeters directly upward to agar in inverted petri dishes. According to the work of Buller (1909), air currents are capable of wafting spores from such a height to the lower leaves. No satisfactory explanation of the distribution from one country to another can be offered as yet. There is no good evidence that the fungus is seed borne.

Jones (1919) has made a careful study of the phenomena of incubation and infection of this fungus in red clover. Germination in the laboratory is not more than 50 per cent, as a rule, even when spores are discharged on plain agar, the best medium for the process. A tube is sent out within twelve hours from any point on the circumference of the spore and passes directly through the cuticle into the leaf. Epidermal and palisade cells resist disintegration until hyphae become very numerous. Germ tubes may enter uncongenial suscepts such as yellow trefoil but cause only a slight yellowing before the germ tubes die. Red- and alsike-clover leaves inoculated at Ithaca in the greenhouse showed very minute brown spots within four days. These enlarged up to 1 millimeter within a fortnight, at which time severely infected leaves were dead.

The secondary cycles follow each other at about three-weeks intervals.

Epiphytology

The disease seems to be favored by wet weather. For example, a thick stand of clover in a field near Kingston, New York, situated in a creek bottom on good soil, was very severely affected in June, 1928. The grower attributed the unusual dying of the lower leaves on his plants to the very wet spring, but an examination showed that *P. trifolii* was attacking the leaves heavily. The spots, which were the largest that the writer had ever seen, contained large amber apothecia that were discharging their ascospores in abundance. Undoubtedly this disease played the chief rôle in killing the leaves. Water is highly essential for the discharge of the ascospores and for their dissemination.

It appears also that the disease is more severe under cool conditions. It is most destructive in New York on the first cutting, but this may be due in part to the more humid conditions prevailing during that stage of growth. Another bit of evidence in favor of the cool-weather hypothesis is that none of the Southern States except Virginia and Tennessee have reported the disease. These two States, especially in the mountainous sections, are cooler than the other Southern States. Jones (1919:18) found that spores are produced more abundantly below 18° C. than above that point. If this condition holds for the field, then the disease should be expected to occur more commonly in the Northern States and even there to be worse in the early months of the growing season.

Control

The control of this important disease is an enticing problem which is still to be solved.

Miss Massee (1914:66 67) in England advocates disinfestation of seed with hydrogen peroxide, since she maintains that the fungus is carried on trash with the seed and even on the seed itself. Jones (1919:35) was unable to prevent the introduction of the fungus into fields by seed treatment.

In view of the evidence above as to the suscept specificity of the Pseudopoziza pathogenes, Miss Massee's (1914) suggestion to clean up weed species of Trifolium and Medicago is of little value.

Sulfur dust for the control of this disease has been tried by the writer both in the field and in the greenhouse. In field experiments in 1926 eight contiguous plots, each 1 rod by 3 rods in size, separated by alleys 1 yard wide, were dusted checkerboard fashion with Kolodust, making four dusted and four undusted plots. The dusting was begun on May 22 and was continued at approximately weekly intervals for six weeks. Pseudopeziza leaf spot was the most serious disease on the clover in the plots, so that weight yields may be taken as a fair index of the control of the disease. At harvest time the green weight of clover from each plot was determined to the nearest one-fourth pound. The average of the dusted plots was 331.3 pounds and that of the undusted plots was 315.8 pounds, a difference of 15.5±7.5 pounds as calculated from pairing the contiguous plots. It is obvious that the difference is not significantly great, and, even if it were, it is too small to be of economic importance.

This conclusion was checked by counting all the clover leaves on three scattered areas 1 foot square in each plot. The leaves were divided into four groups: namely, dead, containing more than 10 spots on each leaf, containing less than 10 spots on each leaf, and disease-free. More than 10,000 leaves were examined.

The data thus obtained showed that the dusted plots had 5.3 per cent more disease-free leaves than the undusted plots. This difference is statistically significant, but not being great, it is in harmony with the field weighings.

In 1927 the plots were cut down accidentally by a laborer before yield data could be procured, but a careful examination just before this cutting showed no appreciable difference between the dusted and the undusted plots.

Several attempts to test the fungicidal efficiency of various fungicides in the laboratory were ineffectual because of the difficulty of obtaining satisfactory spore germination. Greenhouse experimentation on control likewise is unsatisfactory because: (1) the quantity of leaves available for inoculation during any one experiment is limited, since each pot of clover requires a bell jar in the top of which the fruiting culture is attached; (2) sporulation is erratic in pure culture, making uniform inoculation almost impossible; and (3) a good method of taking data is unknown. Too many pots would be required for significant yield data. Leaf, leaflet, or plant counts are unreliable because of nonuniformity of inoculation.

The indications available from field work show that sulfur will prove ineffective as a fungicide, but copper-lime dust warrants further tests since a congeneric pathogene of the vine, *P. tracheiphila*, is controlled with bordeaux mixture (Faes and Staehelin, 1927).

The work of Williams (1927) indicates that resistant varieties may offer some possibilities for control.

POWDERY MILDEW OF CLOVER

Suscepts

Salmon in his monograph of the Erysiphaceae of the world (1900:178-181) lists 356 species of plants in 157 genera as susceptible to the pathogene of powdery mildew of clover. This extensive list includes the following species of Trifolium and its near relatives, Medicago and Melilotus: *Medicago falcata, M. lupulina, M. sativa; Melilotus alba, M. officinalis; Trifolium agrarium, T. alpestre, T. arvense, T. filiforme, T. hybridum, T. incarnatum, T. involucratum, T. longipes, T. lupinaster, T. medium, T. minus, T. monanthum, T. montanum, T. moranthum, T. pauciflorum, T. pratense, T. procumbens, T. repens, T. rubens.* Salmon records the fungus also on other legumes, among which may be mentioned 12 species of Vicia, 2 of Phaseolus, and 1 of Pisum. The work of Hammarlund (1925) and of others, discussed later, indicates clearly, however, that little danger of cross infection exists, because of physiologic specialization, each suscept species having its own race of the pathogene.

In New York, the writer has seen the disease on alsike, zigzag, red, and white clovers. No powdery mildew has been collected on Medicago or Melilotus. Moreover, a survey of American writings on meadow-crop diseases has failed to reveal a single record of the fungus on any species of these two genera.

Mains (1923:312) inoculated strains of red clover from many sources in the greenhouse and found that the North American varieties were generally susceptible, while the European showed in general rather high resistance. Another thing noticeable was the presence of resistant individuals in all except three of the varieties tested.

Monteith (1924:63) says, " The Italian clovers which are most susceptible to anthracnose are most resistant to mildew." That European red clovers are resistant to this disease is borne out by the observations of Delwiche (1926) in Minnesota and Noll and Irvin (1926) in Pennsylvania.

The disease

The name *powdery mildew* is so characteristic of the disease that when it is coupled with the name of the suscept, it is sufficiently descriptive. In France the disease is called *le blanc* (Noffray, 1920).

History and range

Sheldon first reported clover powdery mildew in this country in 1908 from West Virginia (Plant Disease Bulletin **6**:53. 1922), and it was recorded occasionally up to 1921. Late in 1921, however, an epiphytotic of powdery mildew on red clover appeared in the East. Professor F. C. Stewart in conversation with the writer states that he saw the disease early in September, 1921, in New York. According to Stakman (1922:243), " one of the noteworthy pathological features of the year was the unusual presence of clover powdery mildew." Reports such as the following poured into Washington in 1922: " clover mildew new disease in all sections of State; powdery mildew of clover very prevalent; abundant." Haskell and Wood in summing up clover diseases for 1922 state (1923:250), " during 1922 it [powdery mildew] was much more widely distributed [than in 1921] and occurred all over eastern United States from South to North and in eastern Canada. . . . . . Apparently, it did not extend beyond the line of the 100th meridian."

The disease in 1923 appeared to spread westward (Haskell, 1924:302) but was less severe than in 1922. In 1924 there was little change in the situation as a whole (Melchers, 1925:181) except that it was more prevalent in some of the Western States, particularly Oregon, Washington, Idaho, Montana, and Utah. This indicates that the eastern form was spreading.

Haskell (1926) summarizing the situation for 1925 says in effect that the appearance of the disease in the Pacific Northwest in 1924 and its increase in 1925 tend to confirm the theory that a new physiologic from of mildew had been introduced into the United States where it was spreading from east to west. By 1925 (Haskell, 1926:369) the disease had been reported from all the States of the Union except Wyoming, Nevada' New Mexico, Arizona, and California, and from some of the provinces of Canada. In 1926 Kirby and Archer (1927:198) report it as becoming of less importance, and Haskell (1928:347) says it was of still less importance in 1927. Reports for the country as a whole have not yet been summarized for 1928, but, if New York may be taken as a criterion, the disease is about as prevalent as in 1927.

Salmon (1900:181-182) lists the distribution in full but he does not differentiate between the various suscepts, so that it is impossible from his records to determine how widely clover is affected in the various countries of the world.

Economic importance

Although opinions differ, probably the majority of the plant-disease-survey collaborators tend to hold with Valleau (Plant Disease Bulletin **6**:11. 1922) that " it is questionable whether or not it is causing serious damage." Edgerton (page 12 of the same bulletin) says that "the mildew reduces the tonnage of the clover crop and will cause the hay to be slightly off color and of poorer quality." Anderson (page 13 of the same bulletin) reports that in parts of Illinois " the disease is so bad . . . . that when the mower goes through a field a white cloud arises on all sides."

Possibly those who believe that no damage is incurred have not observed plants in the later stages of the disease. A large number of leaves are killed and many others are severely injured. During a hot day in late August or early September diseased plants partially wilt because, no doubt, of the rapidly transpiring mildew mycelium which draws the water from the leaves. A considerable amount of damage certainly results from the mildew in an average year.

Anderson (Plant Disease Bulletin **6**:13. 1922) in Illinois states that " those who handle the dried hay complain that the dust results in a stinging sensation in the nose and throat," and Tehon (page 13 of the same bulletin) in the same State says, " We have heard that a disease or trouble similar to heaves has occurred in the southern part of Illinois from feeding of mildewed clover to stock." Farmers have inquired whether the diseased hay is injurious to stock. According to Haskell and Wood (1923:251), " No actual cases of stock poisoning from this cause were found and feeding experiments conducted with horses, cattle, sheep and swine at the Tennessee Experiment Station resulted in no harmful effects." Probably, however, horses subject to heaves should not be fed mildewed clover hay unless it is sprinkled to lay the dust.

As with other forage-crop diseases, estimates of loss are guesses at best. Temple and Jehle (Stakman, 1922:243-244) estimated that in Maryland in 1921 the second crop was reduced by 5 per cent and the entire crop by 2 per cent. In isolated fields in New York the loss to the second crop probably would reach 20 per cent, but for all fields it would not be more than from 1 to 2 per cent in most seasons.

An experiment to be discussed under protection gives some clue as to the damage caused by this disease. The experiment was made in the greenhouse during the mildew period of the 1928 season, and the plants were subjected to no more unnatural conditions than they would have had in the field during the same period. At the end of approximately a month, during which time the plants were continually exposed

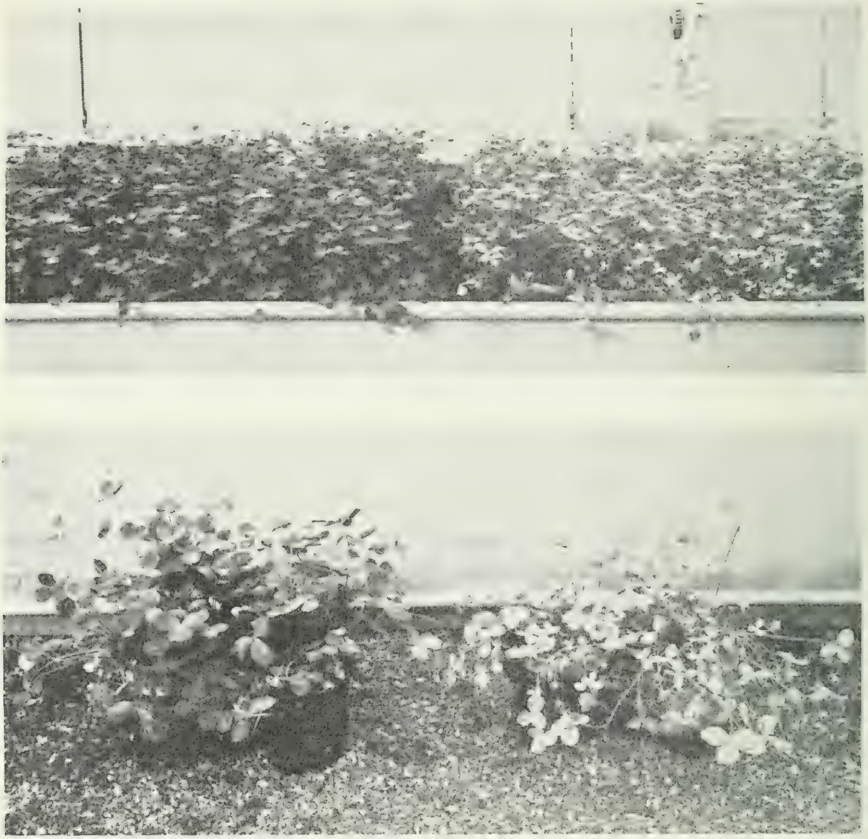

FIGURE 8. EFFECT OF POWDERY MILDEW ON RED CLOVER
The plants on the left were kept healthy by dusting; those on the right were diseased

to inoculation, the average green weight of the dusted plants was 1.74 times as much as that of the undusted ones, and their dry weight was 1.53 times as much. These data appear in table 17 (page 52). Apparently the mildew reduced the crop from one-third to one-fourth. The two photographs in figure 8 show the difference between the two series.

Symptomatology

In New York the disease rarely appears prior to the first cutting. It may occur as early as July 20, but in 1926, 1927, and 1928 it has not developed in abundance until after August 15. The first sign of the disease is a barely perceptible patch of white cobwebby growth on the upper surface of the leaf (figure 9). These patches increase in size, finally coalesce and give the leaf the superficial appearance of having been sprinkled with flour. When a considerable number of leaves are diseased, the whole field appears white from a distance.

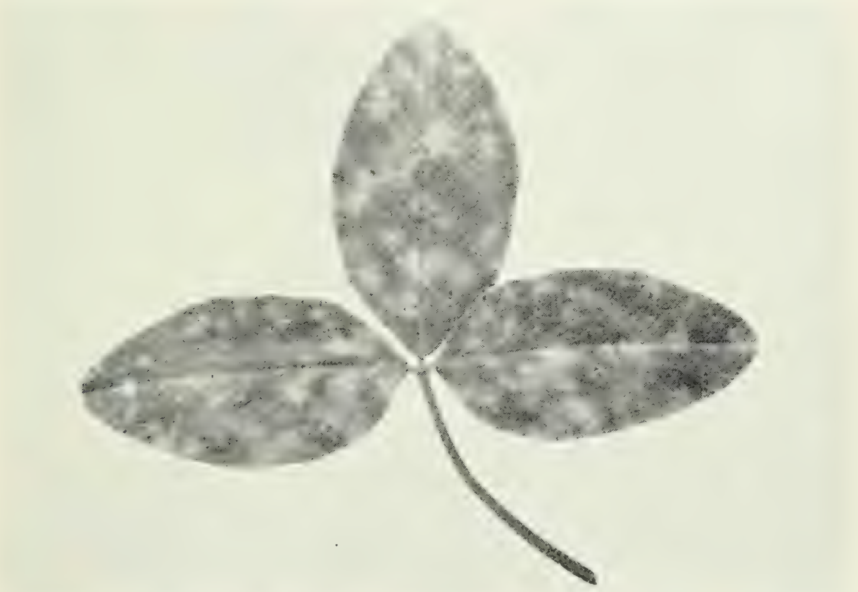

FIGURE 9. A LEAF OF RED CLOVER IN THE EARLY STAGES OF INFECTION BY ERYSIPHE POLYGONI

By the time the mycelium has covered the leaf, the tissue begins to show necrotic effects. Dark purple to black irregular spots appear in severely diseased leaves. These increase in size until the whole blade is dead.

Plants, if diseased when small, are markedly dwarfed. This is evident if mildewed seedlings in the greenhouse are compared with unmildewed ones kept healthy by dusting.

Etiology

Name, history, and classification of the pathogene. Powdery mildew of clover is caused by *Erysiphe polygoni* DC. The perithecial stage has been collected in the western part of the United States but has not yet been found in the East. (Plant Disease Bulletin **6**:53. 1922.)

That the pathogene has long been known is indicated by the fact that the causal organism is one of the few fungi named by Linnaeus, who called it *Mucor Erysiphe* (Systema vegetabilium, ed. 13, p. 825. 1774). The present name, *Erysiphe polygoni*, was applied by De Lamarck and De Candolle (Flore Francaise **2**:273. 1815). It is of interest historically to note that *Erysiphe polygoni* is one of the organisms that Neger used in 1902 in one of the early demonstrations of physiologic specialization in the Erysiphaceae.

The fungus is variable, but the presence of from four to eight spores in the ascus separates it definitely from *E. cichoracearum* with which it is most easily confused. The latter has regularly only two spores in the ascus. Because of the variability of the species even on the same plant, an extensive synonomy has grown up (Salmon, 1900:174–178). *Erysiphe communis* Grev. and *E. martii* Lév. are the most commonly used synonyms.

Pathogenicity. That strains of *E. polygoni* highly specialized in their pathogenicity occur was early recognized by Neger (1902), who showed, for example, that conidia of *E. polygoni* from *Trifolium incarnatum* would not infect *T. repens* or *Vicia sepium*, although they would infect the species of Trifolium from which they came. Salmon (1903:290) stimulated by this work, sowed conidia from red clover upon various closely related species and concludes " that the Oidium on *Trifolium pratense* is a ' biologic form ' specially adapted to this species, and incapable of growing not only on species belonging to other genera of the Leguminosae, but also on numerous species,— if not on all other species — within the genus Trifolium itself." He then predicts that " biologic forms " exist on other species of Trifolium. Seventeen years elapsed before this prediction was substantiated by experimental evidence. Searles (1920:279) found indications of " biologic forms " on *Trifolium minus* and *T. hybridum.* Mains (1923:311) confirms Salmon's form on *T. pratense.* Hammarlund (1925) published a monumental piece of work on the specialization of parasitism of *Erysiphe polygoni* and other species. As a result of his experimentation extending over many years, he set up 26 "formae speciales" in this species. Those on legumes are f. sp. *medicaginis sativae* on *Medicago sativa* and *M. falcata;* f. sp. *medicaginis lupulinae* on *M. lupulina;* f. sp. *meliloti* on *Melilotus albus, M. altissimus,* and *M. petitpierreanus;* f. sp. *trifolii repentis* on *Trifolium repens;* f. sp. *trifolii pratensis* on *T. pratensis;* f. sp. *trifolii hybridii* on *T. hybridum;* f. sp. *trifolii agrarii* on *T. agrarium;* f. sp. *viciae* on *Vicia sativa* and other species of Vicia; and f. sp. *pisi* on *Pisum sativum.* Hammarlund predicts, furthermore, that even more forms will be found when those on other species of Trifolium are investigated.

Blumer in the same year (1925), although maintaining that the forms on Pisum, Lathyrus, and Trifolium are distinct, is inclined to the view that the Trifolium mildew is a single form with the various suscept species showing varying degrees of susceptibility. Considerable evidence, on the other hand, presented by the recent epiphytotic in the United States suggests that even Hammarlund's f. sp. *trifolii pratensis* may represent a group of specialized races. Haskell and Wood (1924:250) say, "There are good reasons to suppose, particularly on account of the conspicuous character of the mildew, its apparent invasion of new localities, and because of the fact that our native clovers are susceptible, whereas European ones are resistant, that we have to deal with an introduced parasite which has found conditions especially favorable in the United States." Haskell (1926:369) says further that the appearance of the disease in the Pacific Northwest in 1924 and its increase during 1925 tend to confirm the theory that a new physiologic form of mildew has been introduced into the United States where it has been spreading from east to west.

Life history. A rather careful review of the literature has revealed little regarding the mode of overwintering and dissemination. The scarcity of perithecia in the East and the South militates strongly against the probability that perithecia are significant sources of primary inoculum. It is likely that the mycelium lives over winter in or on the protected leaves that remain green throughout the winter months. This possibility is made more plausible by the work of Klika (1922:75) who shows that, contrary to the general belief, the haustoria of *E. polygoni* may pass through the epidermal cells and penetrate into the cells of the palisade and spongy tissue, and even into the vascular bundles. He says that this process occurs chiefly in the older leaves in the autumn. The mildew occurs very plentifully in the autumn so that many infected leaves would be present to go through the winter. If so, then the question is, why does not the disease become general until after the first cutting? Where does the organism live during spring and early summer?

The writer desires at this point to indicate an apparent analogy between powdery mildew of clover and late blight of potato. In New York, the two diseases have appeared almost simultaneously during 1926, 1927, and 1928. Dr. Charles Chupp in a conversation in the summer of 1928 stated that late blight appeared a little late that year because the nights had been too warm for good germination of the sporangia. Powdery mildew of clover also appeared in epiphytotic form about ten days later in 1928 than in 1927. It appears probable that hold-over mycelium in the overwintered leaves may not be stimulated to form conidia or the conidia may not germinate well except under conditions of cool nights and warm days. It is a common opinion among greenhouse men that powdery mildew of roses is worse where there is a draft, and, according to Salmon (1907), gardeners believe that strawberry powdery mildew becomes more prevalent when a cool period is followed by warm weather.

Some authors (Van Hook, 1906:249; Crawford, 1927:6) maintain that *Erysiphe polygoni* on peas is carried in the seed. This may be true for the clover mildew as well, but no experimental data are available on this point. Infected seed might readily explain the widespread appearance of the apparently new strain of powdery mildew over the United States during 1921 and 1922.

In 1927 an attempt was made to germinate conidia in aerated distilled water in the laboratory; it was a dismal failure, for only a few short or otherwise distorted germ tubes were obtained. The spore material for subsequent experimentation was obtained by washing the conidia from the mildewed leaves into Syracuse watch glasses with a gentle stream of water from a wash bottle, to minimize injury and to get only the most mature spores. The spores were then transferred with a platinum loop to small drops of water on chemically clean slides which in duplicate were placed in moist chambers at room temperature and were left for thirty hours.

In one experiment conidia dusted dry on duplicate slides and placed in moist chambers were compared with conidia in drops of aerated distilled water. A small difference in favor of wet slides was noted, but, even under wet conditions, the germination hardly exceeded 10 per cent.

Since many chemicals are reputed to stimulate the germination of refractory spores, an experiment was laid out to test the effect of these upon the germination of the conidia of *E. polygoni*. The following treatments were used: (1) a quantity of spores were placed in 1-per-cent citric acid and were removed at five-minute intervals; (2) spores were exposed to ether in a moist chamber for ten minutes; and (3) spores were germinated directly in the following solutions: 0.5-per-cent ethyl alcohol, buffer solutions of pH 5 and pH 7.2, clover extract, distilled water with clover leaves in a moist chamber, and distilled water alone. All the moist chambers were held under identical conditions in the laboratory. The germination in most of the solutions, including the buffer solutions, ether, alcohol, monobasic potassium phosphate, and in water (checks), was approximately 5 per cent, the same percentage as in the preceding experiments. The average germination of the spores in the clover extract on two slides was 19 per cent. Apparently the clover material stimulates the germination. The effect may be due to carbon dioxide acting on the pH of the water, as shown by Platz, Durrell, and Howe (1927) for *Ustilago zeae*, but this was not investigated further since the stimulation was not great.

The conidia may germinate within twelve hours, usually pushing out a germ tube terminally, sometimes laterally. The germ tubes grow slowly in distilled water and show signs of disintegration after about forty-eight hours. The protoplasm becomes vacuolate and hyaline. In clover extract the tubes become somewhat longer and die somewhat less quickly than in distilled water, but none the less surely. The protoplasm remains in the end of the germ tube. This was observed in one case as the protoplasm flowed from the spore to take up this position in the tube.

The first hint of mycelium on the leaves in the greenhouse may be detected within four days after inoculation. The patches of mycelium increase in diameter and fuse, producing within two weeks small necrotic spots which enlarge, finally coalesce, and kill the leaf.

The most marked pathogenic effect of the mildew fungus on the clover plant is stunting. Some data bearing on a possible explanation of this effect are given in table 17. There was a marked difference between the size of the mildewed plants and the unmildewed ones when they were harvested. A glance at table 17 reveals that the disease-free plants averaged 1.73 times heavier than the diseased ones when green and 1.53 times heavier when dry. It appears therefore that the mildew exercises a profound deleterious effect upon the economy of the clover plants. The decrease of the ratio between the two with drying indicates clearly that the diseased plants contain less water than the healthy ones, both upon an absolute and a per-gram-dry-matter basis. This is borne out by the data in table 17 which show that the treated plants average 5.39 grams of water per gram of dry matter and the untreated averaged 4.65 grams. The conclusion obviously is that the untreated plants were living under physiologically dryer conditions than were the treated plants.

Professor H. H. Whetzel in conversation with the writer has suggested that the stunting of mildewed plants is due to the dry condition that has been shown to exist within the tissue and that this dry condition is due to excessive transpiration of the diseased plants. The water is lost rapidly from them through the thick mat of hyphae spread over the leaf. These cylindrical hyphae expose much more surface to the dry air than does the flat leaf surface they cover, even if that were uncuticularized and free of hyphae. They lose water so rapidly that they can hardly be removed from the leaf quickly enough for microscopical examination without shrivelling.

It is well known, of course, that high transpiration causes the following effects: the stomates tend to remain closed, keeping out CO_2; the dry walls of the substomatal chambers pass but little CO_2 and O_2; water for the production of carbohydrates is scarce; and the process of changing sugars to starch tends to reverse itself. The ultimate effect can be nothing but an undersized, scrawny plant.

The results of this experiment support this transpiration theory, because it was noted several times during the course of the experiment, which ran during hot weather, that the mildewed plants wilted very soon if the gardener was a trifle lax with the watering hose. It appears that they lost water more rapidly than the unmildewed plants.

Conidia produced in the primary cycle constitute the inoculum for the secondary cycles which follow each other at less than two-weeks intervals, because the time from inoculation to spore production and further inoculation usually is not longer than about ten days under summer conditions.

Foex (1921) describes the formation of conidia. He says that the vegetative hypha swells just below a nucleus; the nucleus divides and a septum is laid down. The upper nucleus divides again, and a septum separating these two daughter nuclei is laid down, the upper cell becoming a conidium. The lower cell becomes a spore mother cell from which conidia are cut off in succession. The conidiophores of this species are commonly said to be single-spored, but Salmon (1903:265) points out that chains of from 4 to 8 spores may occur. These chains usually escape detection because the spores separate immediately upon mounting in water.

The factors governing the formation of perithecia would be of interest because of a possible explanation of their absence in the East. The opinion is commonly held as a result of Kleb's work (1900) that starvation, or a lack of nutrients, stimulates reproduction in fungi. Klika (1922:75) maintains that, " Die Entwicklung der Perithezien wird entweder durch das Sinken oder ein Schwanken des Substratnährwertes verursacht." Possibly the report from the State of Washington (Haskell, 1926:369) that perithecia of clover powdery mildew were collected on alsike and white clovers is merely another indication of the same tendency, especially if Blumer's (1925) contention is true that these clovers are resistant to the *trifolii-pratense* form and do not harbor separate forms themselves. Fromme and Wingard (1921:392) found that the uredinial generation was frequently elided in varieties of beans resistant to rust. The telial sori appeared instead.

Epiphytology

As has been indicated under inoculation in the primary cycle, the cool nights and warm days of middle and late summer seem to have a marked effect upon the spread of mildew. Wind plays a significant rôle in the summer dissemination of the conidia which are very light and easily blown about. To keep clover plants uninfected in the greenhouse in the autumn is difficult because wind-blown conidia filter in through the ventilators and doors. Moisture is of less importance in the development of this disease than in some others because the conidia of *E. polygoni* will germinate on a dry glass slide in a humid atmosphere almost as well as in a drop of water.

Control

Much of the prevailing opinion regarding control of clover powdery mildew is summed up in a statement by Young (1923:160) when he says, " Practically nothing can be done to control clover mildew under field conditions." Mains (1923:308) holds that " control of a disease of the nature of mildew under the cultural conditions necessary for clover is extremely difficult."

Unless the fungus occurs in the seed, as Crawford (1927:6) has indicated for the pea mildew, then exclusion measures would be useless. Crawford obtained control of the primary infections by treating the seed in hot water at 56° C. for twenty minutes. Even in that case secondary infections soon overwhelmed his plants and the disease became as bad as if he had not treated the seed.

The epiphytic nature of powdery mildew renders it highly sensitive to chemicals applied to the plant. In the greenhouse on two occasions severely diseased red-clover plants in pots or flats were divided into two lots, one of which was dusted twice at two-weeks intervals with Kolodust. Within a month the dusted plants were entirely free of infection and showing a dark, healthy green as contrasted with a sickly yellow and brown appearance of the undusted lot.

Sulfur dust has been almost universally recommended for the eradication of powdery mildews of various suscepts. The use of this material in such a way extends back many years into phytopathological history. What appears to be the first attempt at controlling the disease on clover in the field was reported by Barss (1926) in Oregon. He states that two county agents sprayed clover fields with lime sulfur, 1½ gallons in 100 gallons of water for each acre of clover, and found that the mildew disappeared in five days. There was an increase of 2½ bushels of seed to the acre at a cost of about $1 an acre. Fifteen pounds of sulfur dust to the acre also gave satisfactory control. Barss says, " Hot weather is believed to be essential in getting a thorough control with sulfur dust since the heat causes the sulfur to volatilize and give off fumes which kill the fungus and its spores." This is improbable, as is pointed out in the discussion of the Heterosporium leaf spot of timothy, where it is shown that temperature seems to exert little effect upon the fungicidal action of sulfur. It is more probable that the germinating conidia exercise a solvent action on the sulfur, as suggested by the work of Marsh (1929).

If sulfur dust will eradicate clover mildew once it is established, it certainly should prevent initial infections. An experiment in protecting seedlings was carried out in the following manner in the greenhouse during the mildew period of 1928. Soil of uniform composition was placed in 156 four-inch pots which were planted uniformly to medium red clover on August 9. The plants had become well established by September 6, when the first natural infections were visible. On that date the pots were divided into two groups of 78 pots each. One group was dusted with Kolodust and the other was left untreated as a check. Sixteen pots of heavily mildewed clover plants equally spaced around the experimental pots served, with other mildewed plants about the greenhouse, to insure an ever-ready and a copious supply of inoculum, which eliminated the necessity of applying conidia artificially. A subsequent application of Kolodust on September 12 and another on September 24 kept the treated plants practically free of infection. On October 9 the plants from each pot were cut at the ground line, weighed green, and then weighed again after drying for two weeks at 105°C. The data procured are given in table 17.

TABLE 17. Effect of Kolodust in Protecting Clover against Erysiphe Polygoni in the Greenhouse

	Weight in grams, per pot			
	Dusted		Undusted	
	Total	Average	Total	Average
Green plants	1,042.3	13.36	604	7.74
Dry plants	162.83	2.09	106.73	1.37
Water	879.46	11.27	597.26	6.37
Water per gram of dry matter	......	5.39	......	4.65

Obviously the sulfur controlled the disease, because the dusted plants averaged 1.73 times as heavy as the undusted when green and 1.53 times as heavy when dry. That means that the dusted gave an average of one and one-half times as much dry hay as did the checks.

Differences in resistance have only been suggested thus far, but crosses between resistant European clovers that are susceptible to anthracnose and winterkilling and American strains that are resistant to the latter two maladies and susceptible to powdery mildew might give desirable plants that would be satisfactory from all points of view. Mains (1923:312) states that individuals resistant to mildew exist even among native clover varieties. Selections from these also should produce desirable strains.

CERCOSPORA LEAF SPOT

Suscepts

The writer (1929) has shown that red, zigzag, alsike, white, sweet, and hop clovers, yellow trefoil, and alfalfa are affected with Cercospora leaf spot in New York. Records of the following additional suscepts are recorded in the literature: *Trifolium alpestre* (Saccardo, 1882:556), *T. incarnatum* (Chester, 1890:96), *T. rubrum* (Passerini, 1877:124), *Medicago arabica* (Ranojevic, 1914:420), *M. denticulata* (Ellis and Everhart, 1891), and *M. hispida* (Wolf, 1916:301).

The disease

History and range

This disease is widely distributed in the United States. It was first reported from New York by Peck in 1883. It is also recorded from New Jersey (Ellis and Everhart, 1888:7), Delaware (Chester, 1890:96), Alabama, Iowa, and Texas (Ellis and Everhart, 1891:89), Mississippi (Tracy and Earle, 1895 b:119), Indiana and Michigan (Stakman, 1922:248), Georgia (Haskell, 1926:366), Missouri and Kansas (Kirby and Archer, 1927:197), Utah (Haskell, 1928), and North Carolina (Wolf and Lehman, 1924). The European distribution is indicated by records from Servia (Ranojevic, 1914:420), Italy (Passerini, 1877:124), Austria (Magnus, 1905:558), and Hungary (Penzes, 1927). There is no record of its occurrence in Asia, Africa, or Australia.

Symptomatology

The lesions produced by Cercospora leaf spot are almost without exception somewhat angular and more or less delimited by the veins of the leaf.

The spots on red clover (figure 10, A) and on hop clover are linear and sharply delimited. Terminal lesions on the leaflets are triangular, being limited on either side by veins from the midrib. Sometimes lesions occur on the pulvini of the leaflets, causing them to yellow and fall.

The elongated spots on red clover are in contrast with the almost circular spots on sweet clover, and almost all intergrading conditions are found on the other suscepts. Lesions on sweet clover may be 5 millimeters in diameter. As a rule, alfalfa bears spots of the same general shape as those on sweet clover but smaller in size. In the spring of 1928 near Cairo, Greene County, New York, secondary shoots of alfalfa developing under moist conditions before the first crop was cut showed elongated lesions precisely like those illustrated on red clover. It appears then that atmospheric conditions and the type of tissue influence the shape of the spot. Alsike, white clover, and yellow trefoil have spots intermediate in shape between the circular ones on sweet clover and the linear ones on red clover.

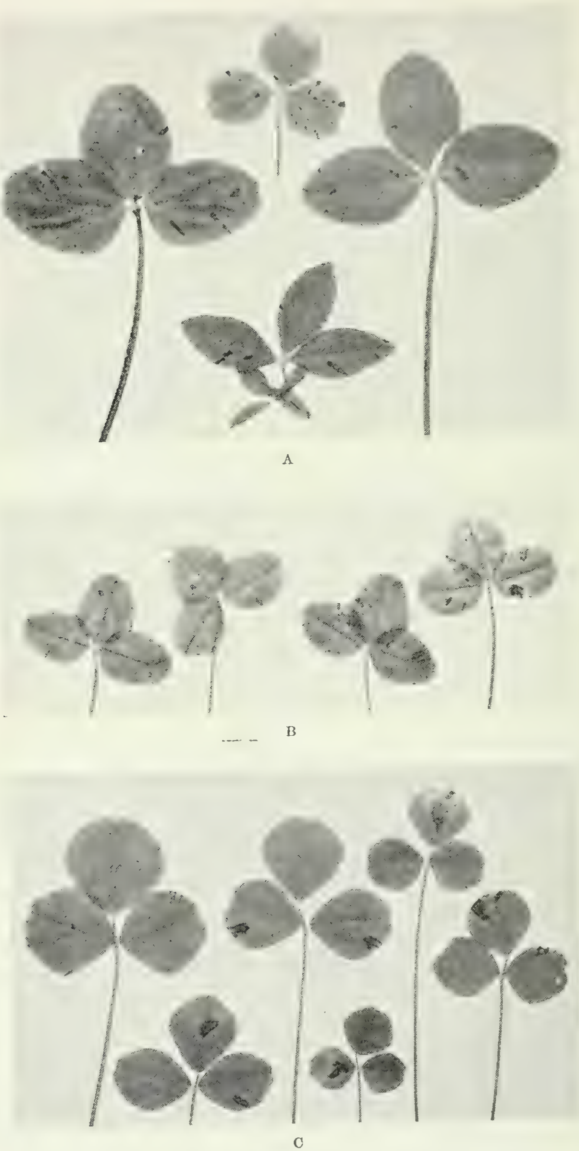

FIGURE 10. LESIONS CAUSED BY CERCOSPORA ZEBRINA ON LEAVES OF RED CLOVER (A), WHITE CLOVER (B), AND YELLOW TREFOIL (C)

These spots are limited by veins to a large extent and are somewhat elongate but not sufficiently to form definitely linear lesions (figure 10, B and C).

Although the color of the spots varies considerably, the general tone is reddish or smoky brown. Comparisons with Ridgway's (1912) color standards show the lesions to be chocolate or warm sepia on sweet clover; olive brown to wood brown on hop clover; deep brownish drab, light seal brown, and Hay's brown on yellow trefoil; bister, seal brown, bone brown, and clove brown on red clover; Rood's brown and Prout's brown on alsike clover; and burnt umber, warm sepia, chocolate, and bister on white clover. The agreement of color symptoms on many of the suscepts suggests the identity of the disease. Not only the shape but also the color of the spots on white clover is intermediate between that on sweet clover and red clover. Warm sepia and chocolate are common to the spots on white and sweet clovers, while bister is common to the spots on white and red clovers. Light seal brown is common to the spots on yellow trefoil and red clover. No fresh material of diseased alfalfa leaves was available for color comparisons, but here also various shades of brown prevail.

The disease occurs on the stems and petioles as somewhat sunken lesions with colors similar to those of the lesions found on leaves. Such stem infections are serious, since they cause the distal parts to wilt and die.

An ashy-gray down on mature spots on leaves and stems is a diagnostic sign of the disease. This down is composed of multitudes of spores and conidiophores covering the surface of the lesion.

Etiology

Name, history, and classification of the pathogene. The writer has given his reasons elsewhere (1929) for referring the fungus that causes this disease to *Cercospora zebrina* Pass. It is sufficient here to state that material from all the suscepts found in this State and from all the specimens available in herbaria agrees even in minute details with exsiccati type material of this fungus collected by Passerini (1887) himself and distributed by Rabenhorst. In that paper the writer lists the following names as synonyms: *C. davisii* E. & E., *C. helvola* Sacc., *C. medicaginis* E. & E., and *C. stolziana* Magnus. All these names have been applied to fungi on the various suscepts collected from time to time since Passerini first described *C. zebrina.*

Pathogenicity. The fungus has been isolated repeatedly in pure culture from single spores. A photograph of a typical petri-dish culture on oat agar appears as figure 11. Alsike and white clovers have been inoculated successfully with conidia collected in the field and with conidia from pure culture. Although the fungus was not reisolated to fullfill Koch's rules of proof, there seems to be little reasonable doubt of its pathogenicity.

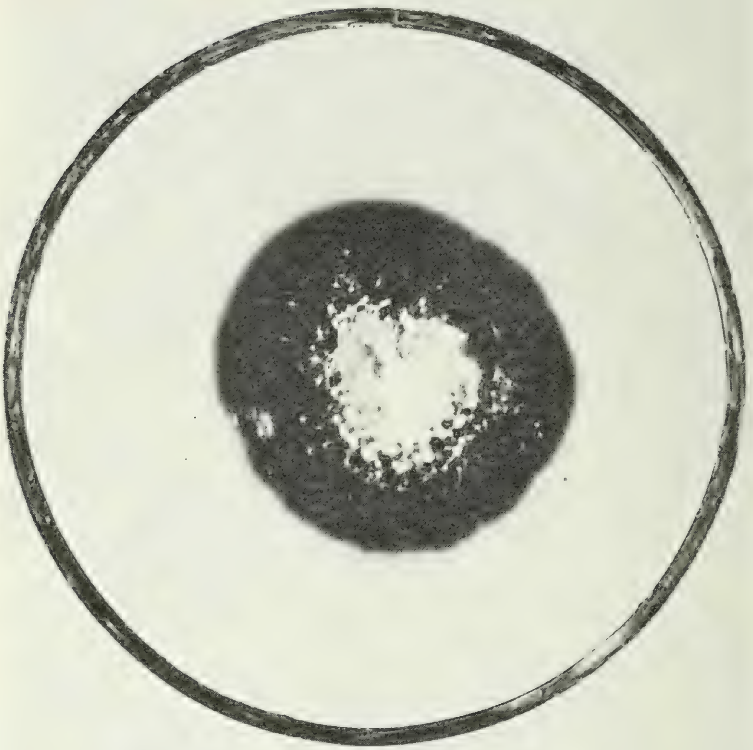

FIGURE 11. A TYPICAL SINGLE SPORE CULTURE OF CERCOSPORA ZEBRINA ON 15 CENTIMETERS OF OAT AGAR

Life history. All the evidence points to seed distribution of the fungus. The author repeatedly has found infections in the greenhouse on primary leaves of alsike and white clover. When seeds from the same lot that produced these infected plants were examined, the superficial mycelium of the fungus, as found by Hopkins (1921) on bur-clover seed, was seen.

Wolf (1916) found conidia in centrifuged washings from seed. Whether these or the seed-borne mycelium or both initiate the primary cycles is uncertain. In any case, conidia produced on the primary lesions are splashed and blown about to initiate secondary infections at intervals of a fortnight or less.

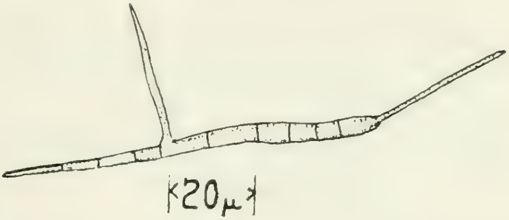

FIGURE 12. A CONIDIUM OF CERCOSPORA ZEBRINA FROM A LEAF OF ALSIKE CLOVER, GERMINATING IN THE PRESENCE OF BANK'S COLLOIDAL SULFUR DUST

No toxic effects are present except possibly a shortening of the tubes

The conidia send forth hyaline germ tubes from one or several cells, as shown in figure 12. Spore-germination studies were limited because conidia ordinarily are not produced in culture. In one experiment, however, spores were washed from an alsike-clover leaf and were germinated in distilled water in duplicate at various temperatures. The data appear in table 18.

TABLE 18. EFFECT OF TEMPERATURE ON CONIDIAL GERMINATION OF CERCOSPORA ZEBRINA

Temperature (degrees C)	Total number of spores	Per cent of germination
3	302	37.8
9	363	88.6
15	362	92.5
21	360	96.3
26	157*	98.1
33	301	77.7

* Only one test.

The counts were made after twenty-three hours had elapsed, so that a fairly sharp curve resulted. If more time had been allowed, the germination doubtless would have been better at the lower temperatures. An optimum at about 25°C. is indicated. An abundant oxygen supply seems necessary for germination. Some fresh spores accidentally left overnight in a drop of water under a cover glass in a moist chamber were examined the next morning. Spores at the edge of the mount were germinated well, with long tubes, but those in the middle were ungerminated. There was a marked gradient of germination toward the center of the cover glass.

Control

Wolf (1916) and Hopkins (1921) recommend exclusion by seed treatment with formaldehyde, 1 pint to 30 gallons of water.

Two laboratory tests to determine the effect of sulfur and of copper dusts on spore germination were made on spores from alsike and hop clover. The data are given in table 19.

TABLE 19. EFFECT OF COPPER-LIME AND SULFUR DUSTS ON CONIDIAL GERMINATION OF CERCOSPORA ZEBRINA FROM HOP CLOVER AND ALSIKE CLOVER

Treatment	Hop clover		Alsike clover	
	Number of spores	Per cent of germination	Number of spores	Per cent of germination
Check	382	90.2	413	98.6
Bank's colloidal sulfur dust	313	33.9	392	68.9
200-mesh dusting sulfur	...	....	403	96.2
7.2-per-cent copper-lime dust	350	0.0	405	2.0

A camera-lucida drawing of a spore germinating in sulfur is shown in figure 12. A drawing of a spore in 200-mesh dusting sulfur would answer for germination in the checks because it was not different.

It would appear, therefore, that copper dusts are more likely to prove valuable in the field than are sulfur dusts, although Kolodust might give some control because of the colloidal sulfur that it contains.

SOOTY BLOTCH OF CLOVER

Suscepts

In New York all three common clovers are affected with sooty blotch. It probably is most common on alsike but scarcely more so than on white clover. Red clover rarely is affected, although two collections have been made, and in each case the attack was severe. Many other species of Trifolium have been recorded as suscepts. These are as follows: *T. agrarium, T. alpestre, T alpinum, T. elegans, T. filiforme, T. fragiferum, T. lupinaster, T. medium, T. montanum, T. reflexum, T. rubens, and T. wormskioldii.*

The disease

The name *sooty blotch* most adequately characterizes the disease. Cooke (1906:247) speaks of the summer condition, *clover black mould,* and the autumn condition, *clover leaf blotch. Sooty spot* is the name commonly applied in the summaries of the *Plant Disease Reporter.*

The disease has been collected in widely separated localities in New York. In the various summaries of the diseases of cereal and forage crops in the United States, this disease has been reported from Minnesota to North Carolina and from Vermont to Washington. Probably it occurs in the United States coextensive with the range of clover. Cooke (1906:247) lists many European countries and Siberia as being within the range of its distribution, and says that it " is not at all uncommon " in England. Estimates in the records of the *Plant Disease Reporter* vary from " injuring 75 per cent of the crop " in Vermont (Haskell, 1919:156) to " 1 per cent injury was estimated " in Delaware (Johnson and Haskell, 1920:73). It has been reported as " one of the most common leaf spots " in North Carolina (Stakman, 1922). Possibly a quotation from Tehon of Illinois will indicate sufficiently well the probable situation with regard to the disease on alsike clover in New York (Haskell and Wood, 1923:255):

> It is a matter of interest to me to find that in the western part of Illinois . . . . the white clover used largely for pasture purposes is covered with the mold. . . . Practically all pastures where this clover is grown are infested. The infection of plants varies from 30 to 70 per cent and from 60 to 70 per cent of the leaves on these plants are affected with a reduction of photosynthetic surface of 2 to 30 per cent.

Killian (1923) maintains that when white clover is attacked only lightly, the malady is somewhat beneficial because the tissue tends to remain green longer. He assumes, of course, in this case that the green tissues function normally, but this is unproved.

Symptomatology

Both dorsal and ventral aspects of diseased leaves of white clover are shown in figure 13. The first signs are minute black pimples which enlarge more or less circularly on the under leaf surface until a vein is

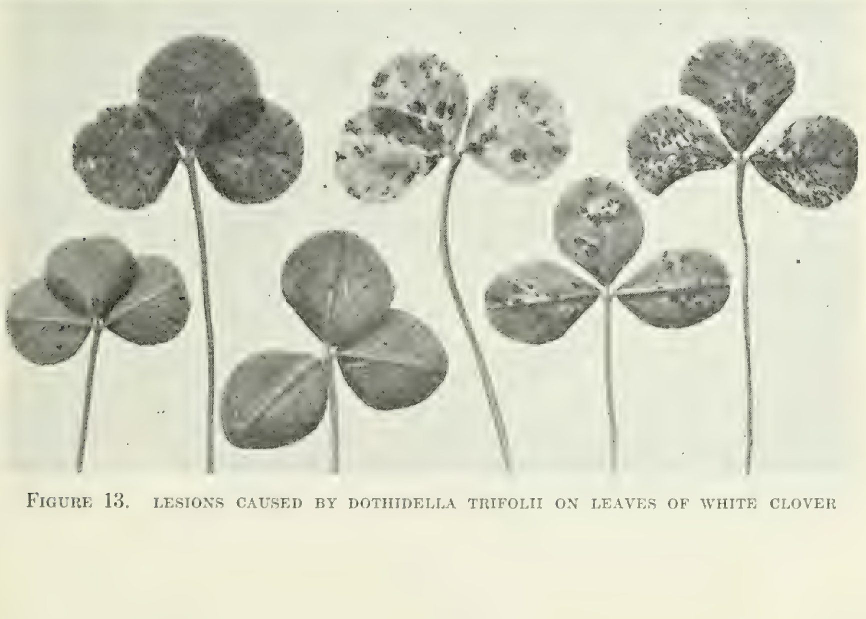

FIGURE 13. LESIONS CAUSED BY DOTHIDELLA TRIFOLII ON LEAVES OF WHITE CLOVER

reached. They then enlarge somewhat and become angular in shape, since the vein seems to limit the radial growth. At this stage the black blotches appear mealy because of the conidiophores and spores that are present. In the autumn these velvety black, somewhat elevated patches are replaced by other black areas which are shiny above but appear warty if examined closely. The warts are the openings of the pycnidia which develop at this stage.

As the black patches, or stromata, grow, the leaf yellows somewhat immediately about them. This is evident on the dorsal side of the leaf directly above each stroma. A casual glance might confuse this aspect of the malady with clover rust. With increasing secondary infections, the yellowed areas spread, finally causing the entire leaf to die and fall off. As already pointed out, Killian (1923) maintains that sometimes, if the infection is slight, the diseased leaves remain green longer than healthy ones. Bayliss-Elliott and Stansfield (1924:219) in England state that " the leaflets of the infected leaves take an upright position and are thus easily distinguished from healthy leaflets which are extended horizontally."

Etiology

Name, history, and classification of the pathogene. Sooty blotch of clover is caused by *Dothidella trifolii* (Fries) Bayliss-Elliott and Stansfield, according to the International code of botanical nomenclature (Briquet, 1912) which stipulates that the first specific name applied to the perfect stage must be used. If the American code be followed, the specific name must be attributed to Persoon. He used it for the pycnidial stage, which he called *Sphaeria trifolii* in 1801 (1801:30). Many years after Persoon's original description, Kunze and Schmidt described the hyphomycetous stage as *Polythrincium trifolii* in Deutsche Schwaemme, fascicle 5, page 5 n. 121, (Greville, 1826). In 1817 they republished the description (1817:13). Fries (1829:368) attributes *Polythrincium trifolii* to Kunze alone.

Nothing of importance happened to the nomenclature of the fungus until 1849 when Fries (1849:368) designated it *Dothidea trifolii*, based on a supposed perfect stage. He does not give any description. Twenty years later Fuckel (1869:218) placed the fungus in *Phyllachora trifolii*, listing *Polythrincium trifolii* as the conidial synonym and the pycnidial stage described as *Sphaeria trifolii* by Persoon as the spermogonial synonym. He lists *Dothidea trifolii* of Fries as another synonym.

When Saccardo (1883:613) listed the fungus, he designated it *Phyllachora trifolii* (Pers.) Fckl., admitting all the while that the " status ascophorus nondum repertus sed habitus omnino *Phyllachorae*,"— a free translation being that although the ascogenous stage had not yet been discovered, the general appearance places it in Phyllachora.

In 1884, M. C. Cooke says he found a specimen in Berkeley's herbarium (1884:63) bearing elliptical, continuous, hyaline spores, 10 to 12μ by 5μ, in clavate asci. Ellis and Everhart (1892:597) state that all the specimens which they have seen in America are immature or sterile. Clevenger (1905:160) in a short paper on North American Phyllachoras reports the collection of a fertile specimen having oval, hyaline, 8 to 10μ by 5 to 6μ, uniseriate ascospores.

Theissen and Sydow in their monograph of the Dothideales (1915:511) refer the specimen described by Clevenger to *P. umbilicata* n. sp. on the basis of differences in the stroma. They retain *P. trifolii* but as a sterile form.

Killian (1923) first saw and reported mature ascospores of this fungus. He wintered the stromata and obtained perithecia with 2 to 4 large fusiform asci containing 8 hyaline, bicellular spores, 26 by 6μ elongated, and slightly curved. Since the genus Phyllachora has continuous spores, he transferred the clover fungus to Plowrightia of the hyalodidymae of the Dothideales. Killian obtained infection with ascospores. By obtaining infection with the spores he demonstrated that the so-called spermogonia are really pycnidia.

A year later, in 1924, two English workers, Bayliss-Elliott and Stansfield, unaware of Killian's work, overwintered stromata that produced perithecia containing asci with 2-celled hyaline spores measuring 24 to 26μ by 7μ to 8μ. The authors point out, in a footnote at the end of their paper, however, that Dothidella Speg. is the correct generic name since it antedates Plowrightia Sacc. A Latin description therefore is given of *Dothidella trifolii* (1924:227).

In the autumn, Bayliss-Elliott and Stansfield found 1-celled hyaline ascospores in their material. This indicates that the spores which Cooke (1884:63) found in Berkeley's herbarium and those which Clevenger found lack the septum because of immaturity. Probably, therefore, *Phyllachora umbilicata* Theiss. & Sydow is a synonym of *Dothidella trifolii* (Fr.) Bayliss-Elliott and Stansfield.

Only one other report of historical interest need be recorded here, that is, *Polythrincium trifolii* var. *platensis* Speg. on *Trifolium platense* (Spegazzini, 1911:437). The word *platensis* appears to be a typographical error.

Pathogenicity. A great many attempts by the writer to culture *D. trifolii* have been made, using a varying technic that included spore dilution in acidified agar containing oat, potato, or clover decoctions and plantings of tissue that had been sterlized in mercuric chloride or calcium hypochlorite for varying lengths of time. Uniform failure has rewarded these efforts. Killian in France (1923) got similar results. Successful inoculations with conidia from leaves have been obtained in the greenhouse. Comparable results have been obtained likewise by Killian (1923) and by Bayliss-Elliott and Stansfield (1924). The two last-named authors, furthermore inoculated plants successfully with pycnospores and ascospores. The fungus undoubtedly is pathogenic, but apparently is an obligate parasite like the rusts.

Life history. According to Killian (1923) the ascospores, matured in perithecia in dead clover leaves in the spring, constitute the inoculum for primary cycles. Conidia from these infections serve as the inoculum for the secondary cycles. Conidia have refused steadfastly to germinate satisfactorily in the laboratory. In two trials no germination whatsoever occurred in aerated distilled water after eighteen hours, but once about 3 per cent germination was obtained in a decoction of clover leaves. Most of the spores sank to the bottom of the drop, although little difference in germination was noted regardless of whether the spores were at the edge or in the center of the drops, indicating that oxygen is not the limiting factor. Germination of conidia in the clover decoction is illustrated in figure 14.

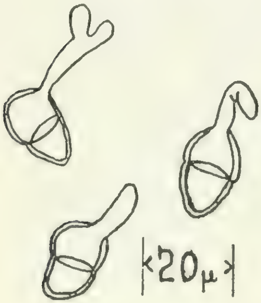

FIGURE 14. CONIDIA OF DOTHIDELLA TRIFOLII EIGHTEEN HOURS AFTER BEING PLACED IN A CLOVER DECOCTION

Note the gnarling of the germ tubes on the spore at the upper left denoting disintegration. Spores placed in distilled water refused to germinate

Killian says that the conidium on the leaf forms a short germ tube which penetrates the cuticle directly at the point of contact with the radial wall between two epidermal cells. Bayliss-Elliott and Stansfield, on the other hand, saw entrance through stomata as well as penetration directly through epidermal cells (1924:221). The mycelium at first invades intercellularly and later intracellularly. Within from six or nine days the black stromata form and push through to the lower leaf surface.

In the autumn conidia cease to form and the stromata containing pycnidia become shiny black and papillate. According to Bayliss-Elliott and Stansfield, as well as Killian, spores from these pycnidia are capable also of initiating infections. It is these stromata which later give rise to perithecia during the course of the winter and thus complete the life cycle of the fungus.

Control

Killian (1923) states that the fungus may live in the soil for five years or more and infect clover crops. If this be true, then attempts at eradication by cultivation or rotation would be in vain. Cooke (1906:247) holds that it would " probably be dispersed by spraying with dilute Bordeaux mixture." On the other hand this seems unlikely since the mycelium of the fungus is almost strictly endophytic, and hence out of reach of external toxic agents.

Killian found that if he planted crimson clover in September rather than in July, as is the usual practice in France, the crop derives a certain amount of protection against the pathogene because the more vigorous early planted crop is more susceptible.

Several attempts to determine the value of fungicidal mixtures as protectants were futile because of poor germination or none at all in the checks. Bordeaux mixture, as suggested by Cooke, or its equivalent, copper-lime dust, might have some effect as a protectant.

PSEUDOPLEA LEAF SPOT

Suscepts

In New York alsike, red, and white clovers, yellow trefoil, and alfalfa are affected by Pseudoplea leaf spot. Yellow trefoil is here reported apparently for the first time as a suscept. *Medicago hispida*, *M. maculata* (Miles, 1925:683), and *M. falcata* (Saccardo, 1902:554) are listed also as suscepts. Alsike and white clover are the most likely to be found diseased in this State.

The disease

This malady occurs widely on the three clovers in New York. It has been collected in Schoharie and Tompkins Counties on alfalfa and in the latter county on yellow trefoil as well. According to the records in the *Plant Disease Reporter* and of Jones (1916:299), it occurs in Georgia, Illinois, Kansas, Minnesota, Missouri, Wisconsin, Alabama, Virginia, Iowa, Indiana, and South Dakota. Doubtless it occurs in other States as well. The European countries from which it has been reported are Denmark (Rostrup, 1899), Wales (Stapledon and others, 1922), Italy (Pollacci, 1902), and Austria (Bubak, 1909). Puttemans (1905) collected it in Brazil.

Symptomatology

The first symptom of the disease on any of its suscepts is the occurrence of hundreds of minute black, somewhat sunken flecks on both surfaces of the leaves and on the petioles, giving them the appearance of having been well peppered (figure 15). Stands of white clover along roadsides actually are darkened at times by the millons of spots on the leaf blades. Attention is called especially to the lesions on the petioles.

On white clover the spots enlarge slowly but rarely attain a diameter greater than from 1 to 2 millimeters, at which time the center is gray with a narrow dark reddish brown zone about the margin. On alfalfa the spots

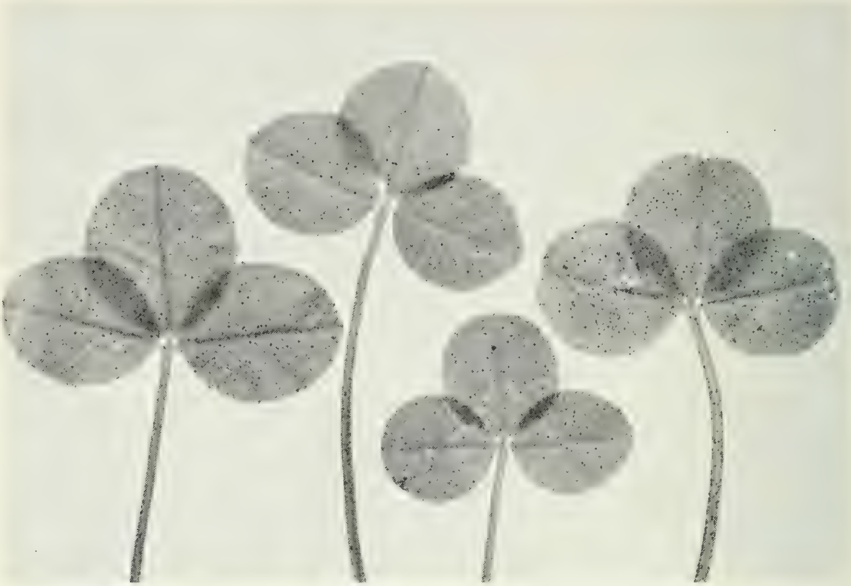

FIGURE 15. LESIONS CAUSED BY PSEUDOPLEA TRIFOLII ON LEAVES OF WHITE CLOVER

may become larger but the colors are about the same. The center of the larger spots tends to crumble away, leaving a ragged edge. Miles (1925: 678) reports lesions on stems, peduncles, calyx, corolla, and even on the seed of *Medicago maculata*.

Leaves may turn yellow and absciss when the lesions become very numerous on the petioles or blades. When this condition obtains to a marked degree in white-clover lawns, the whole stand appears yellow and sickly. Defoliation occurs on all the suscepts.

Etiology

Name, history, and classification of the pathogene. The organism has received several names by different investigators. A very brief review of the history of its nomenclatorial vicissitudes will best serve to present the evidence upon which the writer has based his own decision in the matter. Rostrup (1899) first named it *Sphaerulina trifolii* from white clover in Denmark. Hopkins applied this name to a fungus on red, white, and alsike clovers which he reported for the first time in America in 1923. This fungus was described as having asci bearing eight hyaline, three-septate spores, 30 to 33μ by 12 to 15μ in size. *Pleosphaerulina briosiana* was described on alfalfa by Pollacci from Italy three years later (1902:51). The Italian fungus was first reported in America by Melchers (1915:536), and Jones (1916) a year later confirmed the determination by comparing it with original material. Von Höhnel (1918:162–163) erected a new genus, Pseudoplea, on *Pleosphaerulina briosiana* because he believed the perithecium to be formed in a unilocular stroma and to have no true ostiole. He describes the ascospores as hyaline and muriform.

Three years later Petrak (1921:28–29) removed Pseudoplea from the Pseudosphaeriales where von Höhnel had placed it and transferred it to the Sphaeriales because he maintained that the perithecium had a true wall and a true ostiole. He went one step further and reduced all these names as follows: *Sphaerulina trifolii* Rostrup (1899), *Pleosphaerulina briosiana* Pollacci (1902), *Pleosphaerulina briosiana* Poll. var. *brasiliensis* Puttem. (1905), and *Pseudoplea briosiana* (Poll) v.H. (1918) to synonyms of *Pseudoplea trifolii* (Rostr.) Petrak.

Petrak very probably is correct in combining the organisms on clover and alfalfa under the same species. The only point of separation has been the hyaline spores in clover and the yellow ones in alfalfa and " the universal absence of a longitudinal cross wall in the spores of the clover organism, and the almost invariable presence of the same in the spores of the alfalfa organism " (Miles, 1925:686). Miller (1925:226), however, shows that the spores of the alfalfa fungus become muriform late in their development only and are hyaline when normally discharged, becoming colored only when the light strikes them. The writer, furthermore, has found muriform spores in single spore cultures of the organism isolated from white clover. It appears therefore that spore color and septation are dependent upon the environment rather than upon any innate properties of the organism itself.

Miles (1925) distinguishes the form on *Medicago maculata* as *Pseudoplea medicaginis*, on insufficient grounds as it appears to the writer. The three characters that he stresses in separating it from *Pseudoplea briosiana* and *Sphaerulina trifolii* are: differences in spore size (page 688); the presence of a more evident and a wider sterile border of the thalli on agar cultures, as well as greater abundance of sclerotial bodies (page 681); and on results of inoculation experiments (page 683). The differences between his spore measurements are so small that a mathematical computation of his figures probably would not show them to be significant. The averages, for example, are: alfalfa organism, 31 by 12μ; clover organism, 34 by 13μ; and the bur-clover organism, 35 by 13.5μ. With regard to his inoculation results, it is sufficient to state that the bur-clover organism infected alfalfa and the three species of Trifolium successfully. Conversely, spores of the white-clover organism produced infections on alfalfa and bur clover. The differences in severity of infection which he reports for the three forms on the different suscepts would seem to indicate physiologic races of the fungus rather than distinct species.

In the absence of more definite proof to the contrary, therefore, the causal organism is considered to be *Pseudoplea trifolii* (Rostr.) Petr., and *Pseudoplea medicaginis* Miles is added to the list of synonyms.

Pathogenicity. Results of inoculation experiments reported in the literature are variable. Those of Miles (1925:683) just cited were successful. Hopkins also reports successful inoculation experiments with mycelium from pure culture and with ascospores discharged from perithecia on white clover (1923:124-126). Miller (1925:234), however, denies emphatically that the fungus on alfalfa is pathogenic. He says, "It would seem that under no conditions can the germ tube of this fungus enter the live epidermis of the alfalfa plant, not even when the latter has been mechanically injured." He believes that the fungus is a saprophyte following insect wounds or alfalfa white spot. The writer has made no inoculation experiments with this fungus.

Life history. The factors governing ascospore production have been studied but little. Hopkins (1923:121) obtained no mature perithecia on culture media. Jones (1916:300), however, showed that ripe perithecia and spores could be obtained if cultures are held at low temperatures or out-of-doors in a shady place, especially in the spring and the fall.

The author first obtained spores of the organism isolated from white clover following Jones' procedure. Transfers of the same culture produced mature ascospores on potato and oat agar between April 30 and June 13, 1927, but none between June 13 and July 20 when placed on a north window ledge. The spore production was not different in tubes sealed with paraffin or unsealed. Also, transfers to oat agar on October 20 had produced no mature spores by November 30, but on March 26 the tubes were examined again and mature viable spores were found. The temperature, as recorded by a thermograph beside the tube, once fell to 5° F.

It appears that spores may be produced in culture in spring or winter, but not under the summer conditions of these experiments. A large number of transfers to the same oat agar were made on March 26, 1928. Half of these were covered carefully with black paper to exclude light and then some covered and some uncovered ones were placed in the greenhouse and outside on the same window ledge as was used the previous year. By May 16 the uncovered tubes on the window ledge contained mature ascospores, some of which were muriform. Not a single spore was seen in the tubes from which the light was excluded. On the same date, however, no spores were present in any of the tubes in the greenhouse where the temperatures had been consistently higher. It appears from these experiments that light stimulates spore production in the presence of low temperatures. The nonproduction of spores in midsummer probably is due to the higher temperatures. At any rate, it cannot be due to differences in length of day, because in the last experiment described the length of day was the same in the greenhouse as on the window ledge. Of course, the direct sunlight on the tubes in the greenhouse may have inhibited spore production, although it did not inhibit the growth of the fungus.

It should be mentioned incidentally that the black color commonly found in cultures of the fungus was practically absent from the fungus in the tubes from which the light had been excluded. This agrees with Miller's observation that light causes the darkening of ejected ascospores (1925:226).

Hopkins (1923:125) found perithecia on the dead overwintered leaves in the spring which served as sources of inoculum. Miles (1925:678) finding incipient perithecia on the seed believes that this explains distribution. Miller (1925:226–230) germinated spores after they had lain a year under dry conditions. He found that the asci absorbed water, became inflated, burst the neck from the perithecium, and discharged spores as high as 10 centimeters toward the light. Spores germinate within twelve hours and produce several tubes. These, according to Hopkins, penetrate the cuticle directly (1923:126).

Control

Experiments with fungicides in the laboratory were limited because of a paucity of spore material. Spore suspensions usually were too dilute to be of great value even when the cultures could be induced to form spores at all. The results of one experiment, however, appear in table 20.

TABLE 20. EFFECT OF SULFUR AND COPPER DUSTS ON GERMINATION OF ASCOSPORES OF PSEUDOPLEA TRIFOLII

Treatment	Total number of spores	Per cent of germination
Check	172	95.4
7.2-per-cent copper-lime dust	215	0.0
3.6-per-cent copper-lime dust	80	87.5
Kolodust	89	95.0
200-mesh dusting sulfur	62	98.5
Bank's colloidal sulfur dust	56	93.5

The above experiment illustrates the general reaction of the fungus to copper and sulfur dusts. The spores used came from pure cultures. The fungus apparently is not sensitive to sulfur, so that further work with this protectant looking to field control is likely to be fruitless. Copper-lime dust, however, might give control under field conditions.

CLOVER MOSAIC

Mosaic of clovers is a common virus disease to which little attention as yet has been given by plant pathologists. It undoubtedly deserves careful investigation.

Suscepts

The disease occurs commonly in New York on red, alsike, white, and sweet clovers. It has been reported elsewhere on other suscepts: by Orton on *Trifolium incarnatum* (Fromme, 1921:171); by Mains (1928:363) on *T. arvense*, *T. resupinatum;* by Elliott (1921:148) on *Medicago falcata* and *Vicia faba;* and by Dickson (1922 a:42) on *Medicago lupulina.* Böning (1927) was able to transmit the disease to beans. It is interesting in this connection that Elliott (1921) was unable to get infection on white clover or alfalfa in Arkansas, whereas the virus from sweet clover and red clover easily cross-infected. Dickson (1922 a:42) had no success in transmitting red-clover mosaic to sweet clover in Canada, but white clover readily became diseased with the same inoculum. Dickson (1922 b:88) says, " In one instance there appeared to be a successful inoculation of *Medicago sativa* by means of aphids." In one trial also he succeeded in inoculating *Trifolium hybridum* with juice from *Pisum sativum.*

Mains says (Kirby and Archer, 1927:199) that no varieties of clover are immune to mosaic in Indiana.

The disease

History and range

The earliest reference to a clover mosaic which the writer has found is that by Clinton (1915:360) who said, " Not infrequently the common species of clover show yellowish-green irregular areas." McLarty (1920: 501) asserts that H. T. Güssow saw sweet-clover mosaic as early as 1912 at St. Catherines, Ontario. Several papers appeared in 1920 and 1921 reporting independent observations on the disease (McLarty, 1920; Orton: Fromme, 1921:171; and Elliott, 1921). Dickson published some observations on plants affected and on the symptomatology of the disease in 1922.

Clover mosaic has been reported from Ontario (McLarty, 1920:501) from Quebec (Dickson, 1922 b); from Germany (Böning, 1927); and in the United States from New York west to Montana and south to Louisiana.

Economic importance

Clover mosaic apparently is not a serious disease in this State, although it occurs in most fields in small quantities. In isolated fields it may be really serious in years when plant lice are numerous. The loss comes from the stunting of plants and the reduction in the amount of seed produced as well as from the poor stands that result from low germination of seed from diseased plants (Dickson and McRostie, 1922:42). McLarty (1920: 503) says of sweet-clover mosaic that " when growing wild, diseased plants yield about one-half the herbage of their healthy neighbors." In Pennsylvania, C. R. Orton found a six-and-one-half-acre field of crimson clover in which 50 per cent of the plants were diseased (Johnson and Haskell, 1920). Mains (Kirby and Archer, 1927:199) reports a 0.5 per cent loss for Indiana in 1926.

Symptomatology

To any one who is acquainted with mosaic on other plants, the symptoms of this disease on clover will be familiar. The usual mottled, crinkled, and curled leaves are very evident, as illustrated in figure 16. Frequently the size of the leaf is more reduced than that shown in the photograph. The crinkling is due to an unequal rate of growth between the diseased and healthy tissues, as pointed out by Dickson (1922 b:14). Another hypoplastic symptom is shortening of internodes which, with the dwarfing of the leaves, may at times reduce the size of the plant a half. The inflorescence also is reduced considerably with attendant small yields of seed.

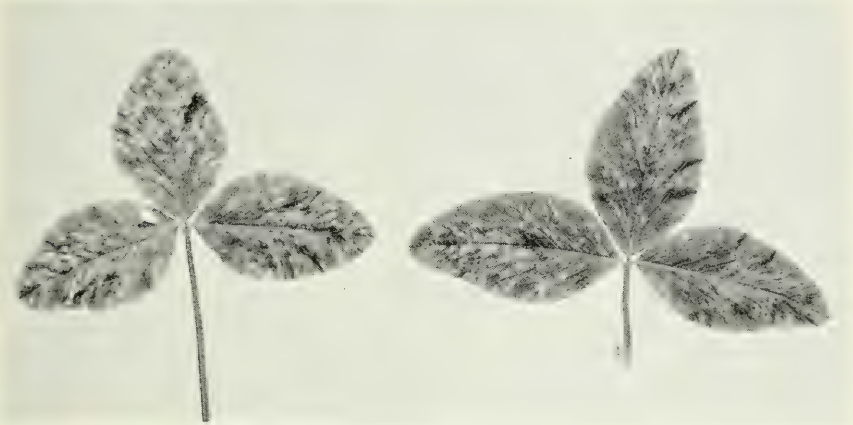

FIGURE 16. MOTTLING AND WRINKLING OF LEAVES OF RED CLOVER RESULTS FROM INFECTION BY MOSAIC VIRUS

Dickson (1922 b:45) states that, " Leaf thickness in [diseased] red clover varies from 105 microns in the lighter to 150–180 in the darker. . . . . . Chloroplasts are fewer and paler in the lighter areas and the intercellular spaces are reduced somewhat by the muriform cell arrangement. In crimson and red clovers the contents of severely infected cells tend to disorganize."

Etiology

Since the causal agents of mosaic diseases remain obscure, a discussion of this feature of clover mosaic does not come within the scope of this paper.

Whatever the nature of the causal agent of clover mosaic, pathogenic effects certainly are produced and the agency is transferable from one clover plant to another. McLarty (1920:503) obtained infection with juice from diseased plants by inoculating it into healthy leaves both before and after passing it through a Chamberland filter. Dickson (1922 a:42) transmitted the disease by means of aphids.

Dickson and McRostie (1922:42) conclude from their experiments on planting seed from diseased red-clover plants that the malady is seed borne and impairs the germination. Seedlings from infected seed or overwintered infected plants serve as sources of primary inoculum. The agents of inoculation in nature are various sucking insects, among which the aphid *Macrosiphum pisi* Kalt. has been shown to be responsible (Dickson and McRostie, 1922:42). Elliott (1921:147) showed that symptoms appear within from ten to fifteen days after inoculation.

According to Johnson (1922), "clover mosaic is seemingly favored by low temperatures."

Control

But little can be offered yet in the way of control. Selection of seed from disease-free fields should be of some value, but obviously roguing diseased plants as practiced for other mosaic diseases is impractical under present conditions of clover-seed production.

KABATIELLA ANTHRACNOSE OF CLOVER

Except for observations on distribution and severity of the disease, the writer has made but little study of Kabatiella anthracnose of clover. The malady frequently is of grave importance in the State. The Colletotrichum anthracnose has been reported from New York but was never collected by the writer.

Suscepts

Red clover only is commonly affected by this disease in New York. The following plants have been reported elsewhere as suscepts: *Trifolium purpureum*, *T. medium* (Malkoff, 1902:283); *T. hybridum* (Fulton, 1910: 752), *T. suaveolens*, *T. repens* (Stapledon and others, 1922:87); *T. in-*

carnatum, *Medicago lupulina*, and *Onobrychis sativa* (Wellensiek, 1926:297). *T. repens* and *T. hybridum*, as well as alfalfa, seem highly resistant if not immune.

In Wales it appears that the late-flowering strains of red clover are less susceptible than are the early flowering ones (Stapledon and others, 1922:85–86). European opinion rates the American clovers high in susceptibility, as indicated in the paper by Westerdijk (1917), whereas in America, the European clovers are said to be more susceptible (Monteith, 1924:63). This point is considered again under the discussion of the distribution of the disease.

Ware, in England, found that Hersnap, a Danish strain, is very resistant (1923:835). Hesler (1925:92–93) points out that the density of stand and the amount of anthracnose vary directly with each other. Tennessee-anthracnose-resistant is reported as susceptible to Kabatiella anthracnose in Kentucky (Haskell, 1928:347).

The disease

Kabatiella anthracnose of clover is an expression logically applied in English because some means of separating the diseases produced by several similar organisms must be devised. It is called " Stengelbrenner " in Germany (Mehner, 1901), " Klaverstengelbrandziekte " in Holland (Wellensiek, 1926), and "scorch " in England (Ware, 1923).

History and range

The first undisputed records of the disease are those of Mehner (1901) and Kirchner (1902) in Germany. According to Stevens and Hall (1921: 331) the disease was first reported in America by Sheldon in 1906 in West Virginia. Stewart (1919:173), however, states that he collected specimens as early as 1903 in New York, The most recent papers dealing with the disease are those of Wellensiek (1926) and Sampson (1928).

Kabatiella anthracnose has a wide distribution. In Europe it occurs in Germany and Bohemia (Linhart, 1902), Switzerland (Stebler and others, 1917), East Prussia (Anonymous, 1926), Bulgaria (Baudyš, 1924), Wales (Stapledon, 1920:637), and Holland (Westerdijk, 1917). It has been reported also from Morocco in Africa (Miège, 1921:39).

The disease occurs generally in New York wherever clover is grown, having been seen in all the counties in which the crop was examined. According to supplements of the *Plant Disease Reporter*, Kabatiella anthracnose has been collected in the United States from Massachusetts to Minnesota and south to Delaware and Missouri. It appears significant that the disease has been collected rarely except in the higher latitudes. The facts pointed out under varietal susceptibility that the southern European varieties seem to be the most susceptible while the northern ones are resistant may be correlated with this. It is also of interest that the Danish strain, Hersnap, was the most resistant in Ware's trials (1923) in England and that the Tennessee-anthracnose-resistant strain was not resistant to this disease in Kentucky (Haskell, 1928:347). If we postulate that this disease is a northern one, then the clovers grown constantly in its presence are more likely to be resistant, whereas clovers not normally subjected to it should be more highly susceptible. A possible explanation for the northern distribution is set forth by Monteith who shows that the causal organism has a relatively low optimum temperature (20° C) for growth, while the minimum temperature is 4° C. (1926:71).

Economic importance

In the three seasons, 1926, 1927, 1928, Kabatiella anthracnose undoubtedly has been the most destructive disease of red clover in New York.

The anthracnose disease is unlike leaf spots in the character of its effects on the suscept. The disease not only kills the tissues actually invaded, but also affects the tissue beyond the infected area as well and frequently kills it. A lesion of a given size on the stem or the petiole will be responsible for much more injury than a lesion of the same unit size on the lamina of a leaf. For this reason the index of infection for this disease obtained in the survey of first-cutting clover in 1928 indicated the disease to be of less importance than either Pseudopeziza leaf spot or Macrosporium leaf spot, whereas it probably is more important than either.

The index of infection in New York in June 1928 was $\frac{(1)\ (9)\ (33)\ (15)\ (42)}{119}$, which means that 7.92 per cent of the stems and petioles of red clover in the State were affected at that time. Inasmuch as the effect of this amount of necrosis on the general economy of the plant is unknown, no estimate of absolute loss can be given. It may be less than 7.92 per cent or more — probably the latter. Estimates in the literature concerning losses are frequent, but they probably mean little except by way of indicating the relative significance of the disease.

Some workers have claimed that the reduction in yield is very large. Lobik (1915), for example, in his study of the effect of fungi upon clover yields reports a reduction of about 50 per cent because of this anthracnose.

Symptomatology

The most diagnostic characteristic of this disease, as pointed out by Kirchner (1902) in his original description of it, is the occurrence of elongated or somewhat stripe-like, sunken, light- to dark-brown spots surrounded by a broad black zone. Sometimes the latter is not prominent, however. These spots are at first small and somewhat watersoaked, but they subsequently enlarge in the direction of the long axis of the stem and become sunken so that at times the pith may be exposed. They may reach from 4 to 5 centimeters by ½ centimeter in size. At such stages the upper part of the stem wilts, turns brown, and dies, giving the field a scorched appearance. Infections on tender petioles or flower pedicles causes these organs to wither and fall over. This also is very characteristic of the disease, especially in the spring when environmental conditions are right. Sometimes the pulvini of the leaves are infected as pointed out by Miss Sampson (Stapledon and others, 1922:83–87), but infections of the lamina are rare. In wet weather the minute white fruiting bodies of the fungus, the acervuli, may be seen with a hand lens in the center of the lesions.

Etiology

Name, history, and classification of the pathogene. Four pathogenes, said to cause clover anthracnoses, have been described. Peck (1880:26) described *Gloeosporium trifolii* in New York as occuring on leaves of red clover. Kirchner (1902:12) twenty-two years later described a species *Gloeosporium caulivorum*, which he distinguished from *G. trifolii* because it occurs exclusively on stems and petioles and because the spores are curved instead of straight. He did not detect any differences in size. Very frequently these two have been considered identical in Europe (Baudyš, 1924, and Voglino, 1909). In America, however, *G. caulivorum* Kirch, usually has been cited as the cause of clover anthracnose.

Bain and Essary (1906 a:193) found still another organism, which they named *Colletotrichum trifolii* because the spores are straight and setae are present in the acervuli. This organism, which seems actually different, has been treated thoroughly in the recent publication of Monteith (1928). O'Gara (1915) distinguishes as new *Colletotrichum destructivum* on *T. pratense* and *T. hybridum* because there are no definite spots, and the spores and setae are much larger than those of *C. trifolii* Bain and Essary.

According to a recent paper by Miss Sampson (1928:104), Karakulin (1923) changed *Gloeosporium caulivorum* Kirch. to *Kabatiella caulivora* (Kirch.) Karak. Miss Sampson states, "My own observations show that it is not a typical Gloeosporium, since it is characterized by a scanty growth of mycelium in the host and on culture media, the absence of a definite stroma beneath the acervulus, and the short club-shaped conidiophore bearing a terminal crown of conidia. These characters agree with those of the genus, Kabatiella Bub., as emended by Karakulin."

The causal agent of clover anthracnose in New York agrees with this description, and it is considered here as being *K. caulivora*. It is sufficient to point out with Baudyš (1924) that the position of the fructification is small ground for separating species, and hence that *G. trifolii* Peck. is probably synonymous with *K. caulivora*. If this should prove to be true, then the name would have to be *K. trifolii* (Peck) Karak. emend. The writer has not seen Peck's specimen.

Pathogenicity. The researches of Mehner (1901:195), Fulton (1910), Wellensiek (1926), and Sampson (1928) have shown beyond reasonable doubt that the fungus is pathogenic to clover plants.

Life history. There has been much controversy regarding the source of inoculum in newly sown fields. The common assumption dating from the paper of Mehner (1901) has been that the fungus is transported with the seed. Fulton (1913:249) says, " The spores when dry retain their vitality for a number of months, and it is likely that such dust-like spores lodged on the outside of seeds may serve to carry infection. Clover trash may harbor the fungus, and serve the same purpose." The point seems still to be in doubt, for Wellensiek (1926:297) says, "As far as exact experiments were undertaken, no carrying by the seed could be observed." Miss Sampson (1928:127) got infected plants by planting inoculated seed in sterile soil under bell jars even though " positive evidence for the transmission of the disease by naturally contaminated seed has not been obtained."

It seems probable from the writer's field observations of anthracnose lesions in the early spring that the fungus overwinters in the perennial green stems. Miss Sampson found a similar condition. Splashing rain undoubtedly is necessary for dislodging the gummy conidia from their acervuli and transporting them to the infection court.

Conidia germinate very rapidly in distilled water, sending out long germ tubes. Miss Sampson (1928:119) placed conidia on succulent stems and studied penetration by stripping off bits of the epidermis at intervals. The cells are penetrated after the third day, but she did not see the actual penetration tube. She assumes that it passes directly through the cuticle. She says that the first evidence of infection is darkening of the epidermal cell immediately beneath the spore. The protoplasm contracts and becomes discolored. Within a week the dark-brown spots appear on the stems on which spores are produced again.

According to Wellensiek (1926:296), " Infection is favored by a temperature of about 20°C. and by great moisture. Drought, on the other hand, seems to make the plants more susceptible."

Control

Many writers, assuming that the organism is carried with the seed, have recommended seed treatment. Baudyš (1924) applied 1-per-cent corrosive sublimate for ten to twenty minutes, but the control was unsatisfactory. Fulton (1913:249) recommends the use of seed from anthracnose-free fields, or seed that has been cleaned mechanically, and disinfection of the seed with formalin solution, 1 ounce to 3 gallons of water, for fifteen minutes. Hegyi (1915) recommends treating the seed with 1-per-cent copper-sulfate solution.

Unfortunately, no data on the sensitiveness of this fungus to fungicides have been obtained because the spores are so small that they are distinguished with great difficulty from the dust particles on slides. This fact makes fungicidal tests in the laboratory difficult and unsatisfactory. It may be said, however, that in the two seasons of field dusting with sulfur no control of anthracnose was apparent in the plots. Apparently this fungus, like *Glomerella rufomaculans* and other anthracnose fungi, is tolerant of sulfur.

The production of resistant varieties is commonly assumed to be the panacea for control of this malady, but no good ones as yet have been discovered. Miss Sampson (1928:111) advances some evidence to show that much of the apparent resistance which has been observed is in reality only klenducity, because propagants that she took from field-resistant and field-susceptible plants were all affected alike when placed under optimum conditions for infection.

STAGONOSPORA LEAF SPOT

The disease for which the name *Stagonospora leaf spot* is proposed, is widespread in this State, occurring most plentifully on alsike clover. It occurs in New York on white, red, alsike, and sweet clovers, as well as on yellow trefoil and alfalfa. It has not been collected as yet on hop clover. Because of a confusion of names of the causal organism, records of the disease are difficult to identify in the literature, but it undoubtedly occurs on other suscepts also.

The lesions, which vary considerably in diameter, are circular or oval to irregular in shape. Diagnosis is made easy by the light-brown to white center and the dark-brown margin of the spots. Comparisons with Ridgway's (1912) color standards give: for the center of the spot, deep olive buff to pallid brownish drab on white clover, white to tawny olive on sweet clover, pale smoke gray on alsike clover; for the margin of the spot, buffy brown on white clover, bister on sweet clover, and brownish drab on alsike clover. In some instances the spots tend to show faint concentric zones of the same color as the margin. These have been observed on all suscepts except sweet clover. This tends to confuse the spot with the Macrosporium leaf spot, but the signs described in the following paragraphs and the papery aspect of the center of the older lesions differentiates the lesions in such cases.

Greenhouse inoculations with single spore cultures isolated from alfalfa, as shown in figure 17, A, result in spots on alfalfa seedlings that are hydrotic at first but later dry and show the dark zones and the dark margins evident in the photograph. The papery character of the central portion of the spots on sweet-clover leaves is shown in figure 17.

The minute dark pimples, the pycnidia, are to be seen prominently in the light-colored centers of the spots. These are characteristic signs of the disease.

The author experienced difficulty at first in identifying the causal fungus. It has been classified under various names according to the age of the spores and the suscept involved. The writer at first identified it on red, alsike, and white clovers as *Phyllosticta trifolii;* on yellow trefoil as *Phyllosticta* sp.; on red clover as *Septoria compta* or *Septoria trifolii;* on alfalfa as *Ascochyta imperfecta*, *A. medicaginis*, or *Stagonospora carpathica;* on sweet clover as *Ascochyta meliloti*, *A. caulicola*, or *Stagonospora meliloti*. Single spore cultures from the Phyllosticta on alsike and white clovers, the Ascochyta on alfalfa, and the Stagonospora from sweet clover, were identical in appearance under the same cultural conditions. The color of the thalli, the yellow color imparted to the medium (oat agar), and the size, shape, and septation of the spores were the same. The fungi agree in every respect with *Stagonospora meliloti* from sweet clover. At the completion of this experiment the writer realized the then very obvious correspondence of the symptomatology on the various suscepts and concluded that the Sphaeroidaceous fungi under consideration on the legumes are probably identical. Subsequently in perusing the literature on *Sphaerulina trifolii*, a paper by Petrak (1919) was discovered in which he has combined all these fungi under the name *Stagonospora meliloti* (Lasch) Petrak. He lists the following names as synonyms: *Sphaeria meliloti* Lasch, *Septoria medicaginis* Desm. et Rob., *Septoria compta* Sacc., *Septoria meliloti* Sacc., *Phleospora trifolii* Cav., *Stagonospora carpathica* Bäumler, *Stagonospora trifolii* Fautr., *Stagonospora trifolii* Ell. & Ev., *Stagonospora dearnessii* Sacc., *Stagonospora medicaginis* v. Höh., *Stagonospora compta* Died., and *Ascochyta caulicola* Laub. This group of synonyms may well include *Septoria trifolii* Ell. & Ev., *Septoria trifolii* Cav., *Ascochyta medicaginis* Bres., *Gloeosporium meliloti* Trelease, *Ascochyta meliloti* (Trel.) Davis, and *Ascochyta lethalis* Ell. & Barth.

Ascochyta imperfecta Peck on alfalfa is certainly different from this fungus which occurs also on alfalfa. The former produces dark purple spots which later become indefinite in outline and yellowed as contrasted with the light-colored spots with a dark margin. Single spore cultures of *A. imperfecta* differ from those of *Stagonospora meliloti* in being almost black on oat agar instead of yellow. Sprague (1929:929) agrees that the two fungi are different, for he says that " there can be little doubt that *A. imperfecta* is different from *A. medicaginis* as shown by inoculation work, coupled with study of material." Apparently Sprague did not

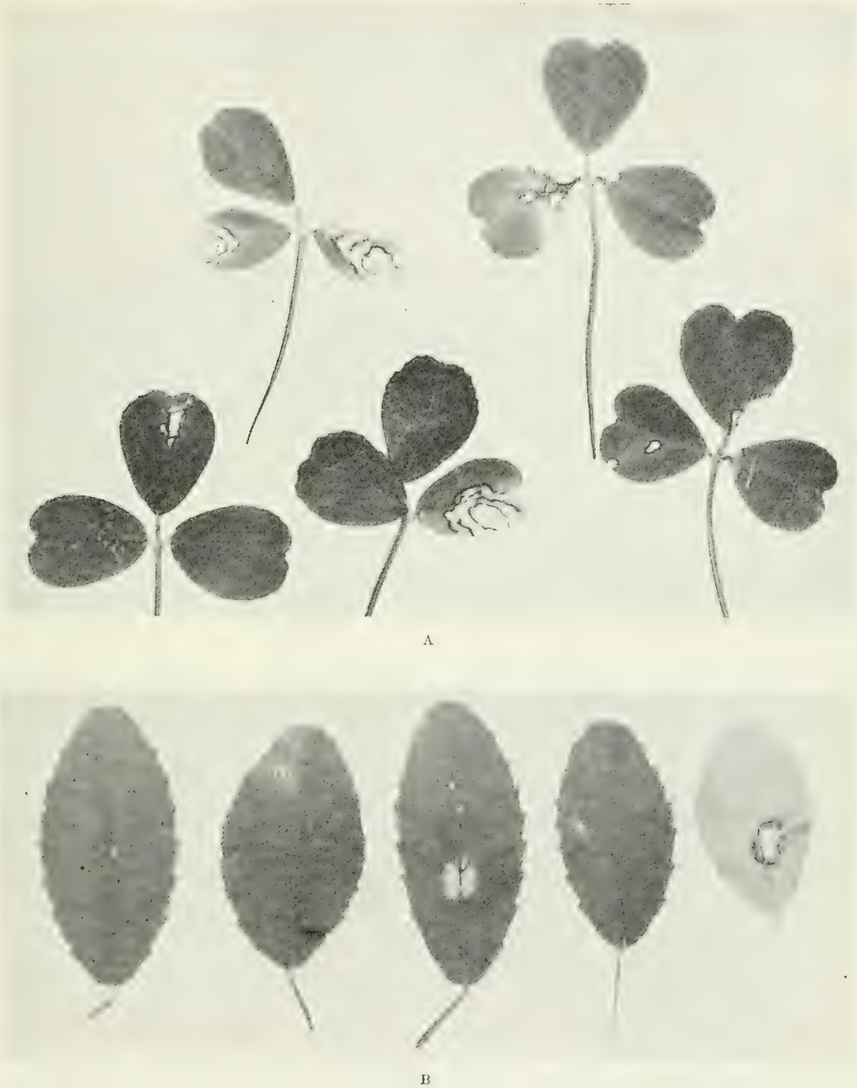

Figure 17. lesions caused by stagonospora meliloti on leaves of alfalfa (a) and sweet clover (b)

collect a fungus similar to *S. meliloti* on alfalfa, for he makes no mention of it.

Little data on the life history or the control of this disease are available. It can be stated that single spore cultures of the fungi from alsike clover and alfalfa have been inoculated successfully onto their respective original suscepts; the disease has been produced in its typical condition; and the pathogenes have been reisolated again. No cross inoculations have been made.

GIRDLE

A peculiar girdle disease of alfalfa, red clover, and sweet clover has been found in the State. It appears to be identical with that described and figured by Brown and Gibson (1922) and apparently has not since been reported outside of Arizona, where it originally was found. The writer found it, however, in August, 1929, near Little Rock, Arkansas, on alfalfa (Plant Disease Reporter **13**:129. 1929). Diseased plants of all the suscepts have the characteristic symptoms of girdle. Leaves on affected stems first turn purplish, later reddish, and finally yellow in color. Stems bearing such leaves are girdled from one to several times by bands of gray-green tissue, bordered on either side by narrow strips of darker colored tissue. Frequently sarcody, or swelling, occurs above the girdle and adventitious roots may begin to grow from the tumid tissues. Apparently no chewing insect is responsible for the injury, because the epidermis over the girdle remains intact. Masses of pink sporodochia of a Fusarium were present on one sweet-clover girdle, but this fungus may have been saprophytic. The Arkansas specimen, a portion of which was deposited in the pathological collections at Washington, D. C., shows pycnidia of what appear to be *Diplodina medicaginis*.

BACTERIAL LEAF SPOT

The bacterial leaf spot, caused by *Bacterium trifoliorum* and described by Jones and others (1923) on various species of Trifolium, was found once on red clover and once on white clover in this State, but it appears to be of little importance.

FUSARIUM ROOT ROT

Various unidentified Fusaria have been isolated during the course of these investigations from rotted clover crowns and roots in the State. Neuweiler (1928) states that *Fusarium trifolii* causes wilt and death of clover plants in Switzerland. Selby and Manns (1909) hold that *Fusarium roseum*, causing the so-called scab of cereals, is able to attack clover plants in Ohio. Young (1924:63) in the same State isolated many Fusaria from rotted clover roots and after making inoculation experiments he concludes that "some of these Fusaria are weak parasites which are able to infect clover but require the cooperation of other deleterious agencies to induce fatal results."

DOWNY MILDEW

Peronospora trifoliorum, the cause of downy mildew, was found once on red clover in the State, but it occurs more extensively on alfalfa (Stewart and others, 1908:394). Patel (1926) found the disease limited to alfalfa and yellow trefoil.

DODDER

Dodder occurs, but it is relatively uncommon on clover and alfalfa. Stewart and others (1908:361 379) give notes on this disease in the State.

RHIZOCTONOSIS

According to Stewart (1919:175), Rhizoctonia causes the leaves of white clover under moist conditions to rot. *Rhizoctonia crocorum* (Pers.) DC. may occur on clovers and alfalfa in the State, since it is known to occur here on potatoes (Plant Disease Reporter, Supplement 34:149. 1924) but it has not been seen by the author. Recently Buddin and Wakefield in England (1927) have connected the fungus with *Helicobasidium purpureum* (Tul.) on fairly good evidence. Stevens and Wilson (1912) report a Rhizoctonia as causing a root rot of clover in North Carolina.

SCLEROTINIA STEM CANKER

Sclerotinia stem canker was collected once in New York at Phelps in 1901 by Stewart (1908:387). Gilbert and Bennett (1917), who have published a good American account of this disease, believe that Stewart's fungus is *S. trifoliorum*. The writer made a thorough search for this disease during June, 1928, but discovered no trace of it in this State.

THIELAVIA ROOT ROT

Thielavia basicola has been found to cause root rot on red clover in New York (Burkholder, 1916:104). It sometimes affects clover in Connecticut also, according to Gilbert (1909:20). It was not found by the author during the seasons he has been in the field.

BLACK STEM

Black stem recently reported by Valleau and Fergus (1929) occurs destructively on red clover in New York. It may be mistaken easily for anthracnose, but no fruiting structures of a pathogene have ever been seen. The cause of the disease is unknown.

DISEASES OF GRASSES

HETEROSPORIUM LEAF SPOT OF TIMOTHY

Suscepts

Timothy alone has been found to be a suscept of the Heterosporium leaf spot. Although a similar disease has been observed on red top, it probably is not identical. Gregory (1919:578) states that orchard grass is never infected when inoculated in parallel with timothy. The writer has obtained infection only on timothy in the greenhouse. Inoculations upon Kentucky blue-grass, red top, and orchard grass have uniformly failed of positive results, although timothy in the same series of experiments was always infected.

The disease

The name *Heterosporium leaf spot* is used in this paper to distinguish this from other leaf-spot diseases on timothy. Gregory (1919:576) first applied this name.

History and range

This disease apparently was first described in a short paper by Gregory in 1919. He states that he found timothy plants affected by this disease in many parts of New York, and he is of the opinion that it probably occurs wherever timothy is grown (1919:576). The only foreign report of the disease since its discovery is a doubtful case mentioned by Jørstad (1924:17).

Economic importance

Gregory (1919:576) believes that the actual damage is negligible, but states that this is the most common disease of timothy in New York. The writer has observed several cases in which the disease was so severe that 100 per cent of the plants in the field were more or less spotted. Although the loss from leaf spots is hard to estimate, the damage probably is not negligible.

Symptomatology

Once the identity of the spots, which occur only on leaves, has been recognized, they are readily distinguished thereafter. The spots are small, oval in shape, with the long axis parallel with the veins. They vary from 0.5 to 1 millimeter in width and from 1 to 4 millimeters in length; occasionally they are larger. The center of the spot is characteristically light mauve colored with a narrow dark madder violet or dark nigrosin violet margin (Ridgway, 1912). Sometimes the purple color is faded, so that the margin appears dark brown. At other times the reverse condition obtains, in which case the purple color becomes so dark that it appears almost black. When spots become numerous, the tissue between them is yellowed, and severely affected leaves are killed.

Etiology

Name, history, and classification of the pathogene. The causal agent in this disease is the fungus *Heterosporium phlei* Greg. The original description constitutes almost the only record of the fungus. The reason probably is that the fungus rarely produces spores in the field, and this makes mycological identification difficult. Spores have never been observed in nature by the writer. The fungus is closely related to the genus Helminthosporium, from which it differs in having echinulate conidia, two- to several-septate. Helminthosporium spores are smooth and pluriseptate.

Pathogenicity. Gregory (1919:578) first completed Koch's rules of proof with *Heterosporium phlei.* The results obtained by the writer agree with those of Gregory. Several futile attempts were made to isolate the fungus during the summer of 1926, but it was successfully isolated later by sterilizing bits of affected leaves for five minutes in 1-1000 bichloride of mercury followed by a thorough rinsing in sterile water. Success in isolation depends upon transferring a piece of leaf bearing a spot to a tube of agar rather than planting several on an agar plate as is usually done. Thus any rapidly growing saprophyte can overrun the pathogene only in the tube in which it happens to occur. In the first isolation three tubes out of ten showed this fungus in pure culture at the end of two weeks as a minute outgrowth from the piece of leaf tissue. Single spore cultures were obtained subsequently. Oat agar is colored dark purple like the leaf spots. Inoculation with two strains produced the typical leaf spots in ten days on two-months-old timothy seedlings in the greenhouse. The fungus was reisolated from the lesions, and the typical cultural characters of *Heterosporium phlei* were produced again.

Life history. Almost no data on the life history of this fungus are reported in the literature. It seems to overwinter on the green leaves, as lesions have been observed on these in the early spring before secondary infections could have occurred.

Spores germinate readily in tap or distilled water in the laboratory on glass slides in about twelve hours, sending out a germ tube from one or both ends.

Obviously, the temperature at which spores will germinate best is a factor in incubation. Two experiments to test this were tried, the average results of which are presented in table 21 and illustrated graphically in figure 18.

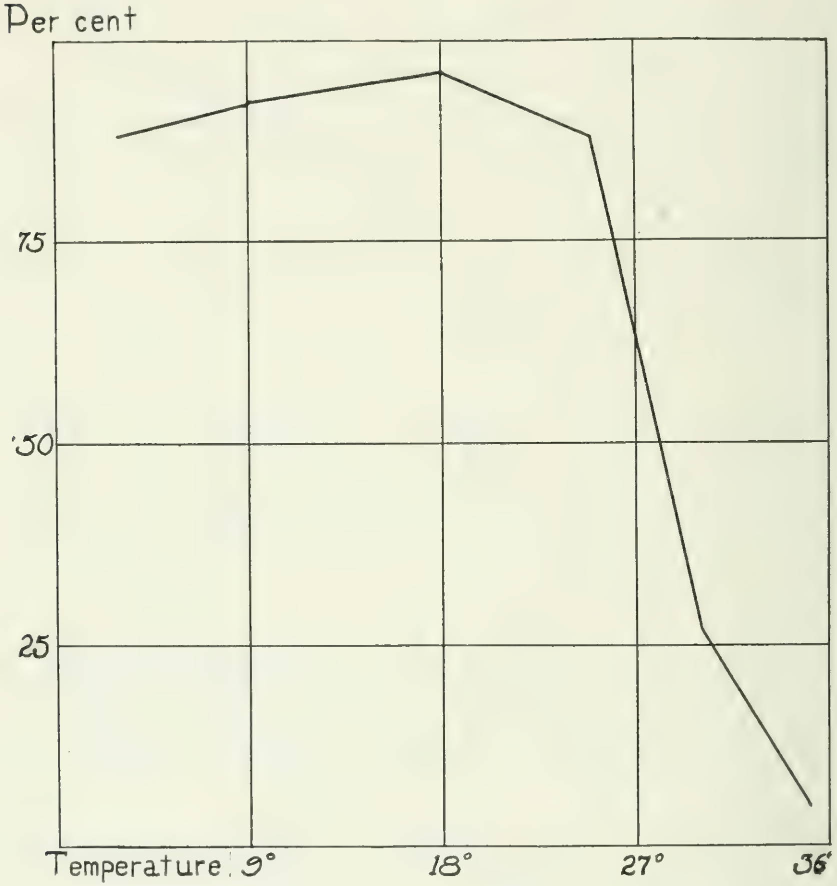

FIGURE 18. EFFECT OF TEMPERATURE ON GERMINATION OF CONIDIA OF HETEROSPORIUM PHLEI

TABLE 21. EFFECT OF TEMPERATURE ON CONIDIAL GERMINATION OF HETEROSPORIUM PHLEI

Temperature (degrees C)	Number of tests	Number of spores	Per cent of germination
3	4	1,049	87.7
9	4	965	92.1
18	2	441	97.1
25	4	948	87.5
30	2	434	27.2
35	2	457	5.0

It is evident that ordinary spring and summer temperatures are satisfactory for germination of the spores, since they seem to germinate practically as well at 3° as at 25° C. Temperatures of 30° and 35° C. appear to be too high for the best germination. More time for germination at the lower temperature levels might have allowed more germination of the spores.

In both cases the spores were allowed to remain on the slides for forty-eight hours before making germination counts. This was to make sure that all the germinable spores had grown; but as time curves for the various temperatures were desirable, another experiment was made to provide the necessary data.

It is shown in figure 18 that germination occurs equally well at 25° and 3° C., but it was predicted that the curve for 3° would show a lag behind the curve for 25°. Accordingly, a series of slides of the same spore suspension was prepared and placed in moist chambers at the two temperatures, 3° and 25°. Two slides were removed from each incubator at intervals of two hours, and the amount of germination was recorded. The results of the experiment are presented in table 22.

TABLE 22. EFFECT OF TIME UPON CONIDIAL GERMINATION OF HETEROSPORIUM PHLEI AT 3° AND 25° CENTIGRADE

Time	Hours elapsed	3° Centigrade		25° Centigrade	
		Total number of spores	Per cent of germination	Total number of spores	Per cent of germination
9 a.m.	0				
11 a.m.	2	No germination		No germination	
1 p.m.	4	448	2.2	580	23.6
3 p.m.	6	426	5.0	459	70.6
5 p.m.	8	262	10.3	491	85.7
7 p.m.	10	503	30.1	481	89.9
9 p.m.	12	445	45.8	446	92.6
10 p.m.	14	501	55.7	450	91.8
8 a.m.	23	541	84.0	473	89.8

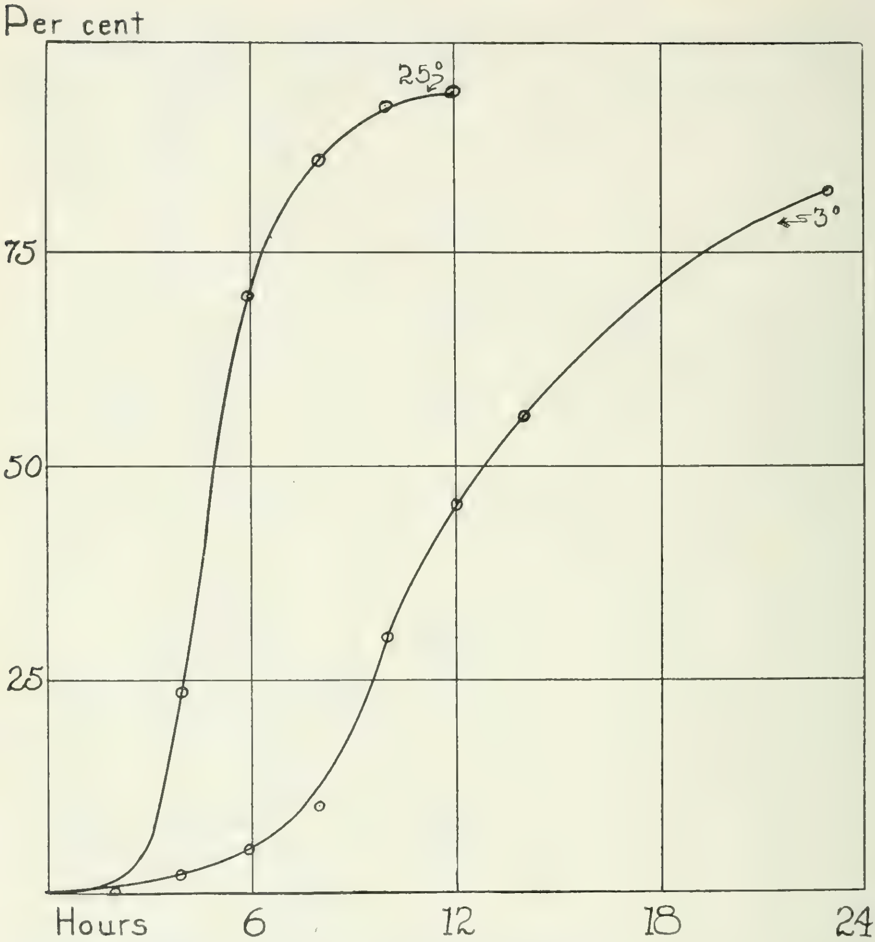

FIGURE 19. GERMINATION OF CONIDIA OF HETEROSPORIUM PHLEI AT 3° AND 25° C., SHOWING GOOD GERMINATION AT THE LOWER TEMPERATURE DESPITE A LAG IN THE CURVE

The results, as shown graphically in figure 19, are exactly those that were predicted. There is a well-defined lag in the germination of the spores at the low temperature.

Nothing is known of the mode of entrance of the germ tubes except thaf they appear to be able to penetrate uninjured tissues.

In one experiment a spore suspension in distilled water was atomized on two-months-old timothy seedlings in pots in the greenhouse, and the pots were placed in a moist chamber for forty-eight hours. Seven days later small light-brown spots had begun to appear on inoculated leaves. Within two days the spots had become more typical, showing the light straw-colored center and the purple margin.

Although no definite data are at hand on secondary cycles, the spores doubtless are carried by rain and wind from plant to plant during the growing season, giving rise to new lesions.

Control

Since the fungus has been shown to germinate practically as well at 3° as at 25° C. if time enough be allowed, it should serve as a most satisfactory organism with which to test the common opinion that the action of sulfur depends upon its volatilization at high temperatures. Barss' statement (1926) that " hot weather is believed to be essential in getting a thorough control with sulfur dust since the heat causes the sulfur to volatilize and give off fumes which kill the fungus and its spores " has been cited already under the discussion of powdery mildew of clover. To test this hypothesis, spores of *H. phlei* were germinated in contact with sulfur in two experiments each in duplicate at various temperatures to see if the toxicity of the sulfur varied with the temperature. The results of these two experiments appear in table 23.

TABLE 23. Effect of Temperature on the Action of Sulfur on Spores of Heterosporium Phlei

Experiment no.	Temperature (degrees C.)	Treatment	Number of spores	Per cent of germination
1	3	Check	455	90.3
		Kolodust	428	5.6
		300-mesh dusting sulfur	419	18.8
	9	Check	417	93.2
		Kolodust	382	12.4
		300-mesh dusting sulfur	417	16.2
	18	Check	451	97.1
		Kolodust	403	11.1
		300-mesh dusting sulfur	389	44.0
	25	Check	422	92.4
		Kolodust	408	7.3
		300-mesh dusting sulfur	464	31.6
2	3	Check	532	83.7
		Kolodust	445	2.9
		300-mesh dusting sulfur	435	37.0
	25	Check	473	89.8
		Kolodust	425	5.4
		300-mesh dusting sulfur	501	31.0

Bank's colloidal sulfur dust prevented germination completely at all temperatures and hence is not included in the table. The differences in germination in Kolodust were not great at any temperature. The same is true of the germination in 300-mesh dusting sulfur, but the latter invariably allowed more germination than the former.

It appears that sulfur is as active at one temperature as at another. Kolodust shows less variability in this respect than does 300-mesh dusting sulfur. This is probably due to the fact that the 300-mesh sulfur is near the border line of toxicity. It has been pointed out previously that dusts at the lower limit of toxicity cause variations in germination in different experiments because of difficulties inherent in the method of application of the dusts.

Caution must be observed in drawing conclusions from the data. One could not say, for example, that sulfur does not kill by volatilizing, but, if volatilization is responsible, it proceeds sufficiently rapidly at 3° C. to inhibit the germination of *Heterosporium phlei*, and that an increase in temperature from 3° C. to 25° C. does not change the degree of toxicity.

If all the germinations in the checks and in the two sulfurs be considered as accurate indications of their true values, then a summary of the results with sulfur is obtained as shown in table 24.

It is readily evident from the table that Kolodust is superior to 300-mesh dusting sulfur in efficiency, but that both are rather effective in

TABLE 24. SUMMARY OF THE EFFECT OF SULFUR ON CONIDIAL GERMINATION OF HETEROSPORIUM PHLEI

Experiment no.	Temperature (degrees C)	Per cent of germination			
		Check	300-mesh dusting sulfur	Kolodust	Bank's colloidal sulfur dust
1	Room	90.1	6.8	11.4	2.7
1a	Room	90.4	3.8	11.5	2.8
2	3°	92.5	18.4	5.8	0.0
2a	3°	88.2	19.2	5.4	0.0
2b	9°	94.6	19.5	10.0	0.0
2c	9°	91.8	12.9	14.9	0.0
2d	18°	98.1	64.5	11.2	0.0
2e	18°	96.2	23.5	11.1	0.0
2f	25°	93.9	40.9	7.1	0.0
2g	25°	90.9	26.3	7.5	Trace
3	3°	81.6	37.4	3.3	...
3a	3°	85.8	36.5	2.4	...
3b	25°	90.0	28.5	5.5	...
3c	25°	89.6	33.6	5.3	...
4	Room	96.3	....	13.1	...
4a	Room	95.9	....	27.5	...

Check, $E_m = 91.6 \pm 0.76$.
Kolodust, $E_m = 9.56 \pm 0.046$.
300-mesh dusting sulfur, $E_m = 26.6 \pm 2.84$.

inhibiting germination. Kolodust probably would give protection against the disease in the field.

TIMOTHY RUST

Suscepts

The results of a great number of cross inoculations between timothy, cereals, and wild grasses reported in the papers of Eriksson (1894), Kern (1909), Arthur (1909), Johnson (1911), Pammel and King (1912), Mercer (1914), Stakman and Jensen (1915), Stakman and Piemeisel (1916 and 1917) have been compiled. The plants shown to be infected more or less readily from inoculations with urediniospores from timothy are: *Avena elatior, Avena fatua, Avena sativa, Bromus tectorum, Dactylis glomerata, Elymus virginicus, Festuca elatior, Hordeum jubatum, Hordeum vulgare, Lolium italicum, Lolium perenne, Poa compressa,* and *Secale cereale.* The following plants have been inoculated by investigators from time to time without positive results: *Agrostis alba, Agropyron occidentale, Elymus canadensis, Elymus robustus, Holcus lanatus, Hystryx hystrix, Phalaris arundinacea, Poa pratensis, Triticum vulgare,* and *Mahonia aquifolium.* As a result of their studies, Stakman and Piemeisel conclude that the following plants may be infected in nature with timothy rust: *Dactylis glomerata, Festuca elatior, Festuca pratensis, Koeleria cristata,* and *Phleum pratense.*

Whether the barberry, *Berberis vulgaris,* is susceptible to the rust is still debatable. Eriksson (1894) did obtain infection once under uncontrolled conditions, but apparently no one has been able to confirm the experiment. As far back as 1864 Settegast observed that timothy can grow very near rusted barberries without becoming diseased (1864:384). It should be pointed out here that timothy is susceptible not only to timothy rust but also to *Puccinia coronata,* oats-form and lolium-form (Melhus, Dietz, and Willey, 1922:225 and 227); and to *Puccinia graminis avenae* (Stakman and Piemeisel, 1916:816) but is not susceptible to *Puccinia graminis agrostis* (Stakman and Piemeisel, 1917:470), to *Puccinia graminis tritici* (Carleton 1899:54, Stakman and Piemeisel, 1917:470), nor to *Puccinia graminis hordei* (Stakman and Jensen, 1915:213).

The general consensus of opinion among investigators seems to be that no timothy varieties are immune to timothy rust, although much resistance is indicated by some of them. Clark (1910:431) observed that "some plants show great susceptibility to the attacks of this fungus, being completely covered with the pustules. On the other hand, a few plants have been observed which are very nearly immune."

As a result of their study of varietal susceptibility, Hayes and Stakman (1919:68) say that "the striking fact is that the Cornell selections show a high percentage of resistant plants, while the Minnesota selections are very susceptible."

The disease

Since the name *timothy rust* is adequate for the purpose, it is adopted in this bulletin.

History and range

Settegast in 1864 remarked that timothy is not affected with rust when growing in juxtaposition to barberries (1864:384). In the United States, Trelease (1885:131) first recorded timothy rust in 1885. It was not reported again in America until 1906 when it " became epidemic in the experimental plats at the Arlington Experimental Farm" (Johnson, 1911:7). In 1907 it was first discovered in New York in the Cornell timothy plantings at Ithaca. It was abundant the same year in Pennsylvania and Indiana (Webber, 1912:352). From that time the fungus has been found in many places in the United States and Canada. Mercer (1914:20) remarks that " the spreading and destructiveness of the disease has been little short of phenomenal."

Arthur (1920:297) gives the distribution of this rust from Nova Scotia and Maine to Louisiana and from Virginia to Washington, California, and Manitoba. The disease appears to be ubiquitous in the United States and Canada. The Sydows (1904:785) report the disease in Europe from Germany, Austria, Denmark, and Sweden. Stapledon and others (1922: 16) find it in Wales, and Madame Teterevnikova (1927) states that it occurs in a severe form on timothy in Russia.

Economic importance

Timothy rust is a destructive disease. Webber (1912:352) in an early report on the disease in New York State says, " Observations made every year for the last five years in various parts of the State have shown that rust is the most serious disease affecting timothy." During 1926 to 1928, however, this condition did not obtain in New York. It may be that timothy was more subject to the disease when it first became epiphytotic during the first decade of this century. At any rate, it was far overshadowed by the Cercospora leaf spot in 1928. Miss Sampson reports a great reduction in seed yield in Wales as a result of rust. She says that in 1922 the weight of 1000 seeds was reduced from 0.39 to 0.19 gram and in 1923 the weight of an equal number of seeds was reduced from 0.43 to 0.31 gram. Johnson and Haskell (1920:75) report an estimated loss of 2 per cent of the crop in Minnesota in 1919. Cook and Helyar (1915:6) note that " the timothy rust is also occasionally found in New Jersey but cannot be considered of importance."

Symptomatology

Symptoms do not play a very important part in the diagnosis of this disease. The leaves may be of a nondescript yellow color as they are killed by copious infections, but when only one or two small infections occur scattered over the leaf, little or no discoloration appears adjacent to them. Sometimes there is a narrow zone of yellow surrounding each pustule. Barker and Hayes (1924:367) note that " very sharp hypersensitive areas do not appear in timothy. Resistant plants give small pustules which are not confluent. Marked chlorosis is usually, but not always, associated with resistance."

Timothy rust is most noticeably evident on leaves and culms in the urediniospore stage as yellow-brown, characteristically elongated pustules protruding through the epidermis which is visible with a hand lens as a whitish partially sloughed off scale. The oblong linear sori may approach 5 millimeters in length. In some instances they are confluent. The uredinial sori on timothy culms are shown in figure 20. Note the scurfy appearance due to the bits of epidermis which have been pushed aside by the developing sori.

Etiology

Name, history, and classification of the pathogene. Even after more than twenty-five years the question that Eriksson asked in 1902 seems to persist, " Ist der Timotheengrasrost eine selbständige Rostart oder nicht?" In 1894 Eriksson and Henning (1894:140) described a new species, *Puccinia phlei-pratensis*, on timothy, differentiating it on the basis of the lack of an aecial stage on Berberis. In 1902 Eriksson asked the question just quoted and maintained that it should be a separate species. He thinks (1902:198) that *Puccinia phlei-pratensis* may have arisen originally from *Puccinia graminis* and " that it has differentiated gradually over a long period of time into a distinct species, in so far that it has lost its aecium-producing capacity, but that it has retained its inner nature so that it can return weakly to oats and rye " Klebahn (1904:235), Sydow and Sydow (1904:784), and Evans (1907:447) likewise retain Eriksson's name.

Kern (1909) maintains that Eriksson's one successful inoculation of barberry is worth more than all the negative results since obtained, so that he advocates calling the fungus a physiological form of *Puccinia poculiformis (graminis)*. A year later (1910:418), however, he retracts somewhat and says, " Rather than calling it a race, physiological species or form species, it might be better to consider it a variety or subspecies since it does, as previously pointed out, possess some slight morphological differences from the typical form, particularly in the small aecial cups and the more delicate uredinial mycelium." Johnson (1911), Pammel and King (1912), and Mercer (1914) use the name *Puccinia phlei-pratensis*. Stakman and Jensen (1915) avoid naming the fungus in their paper reporting inoculation experiments, but Stakman and Piemeisel (1916) call it

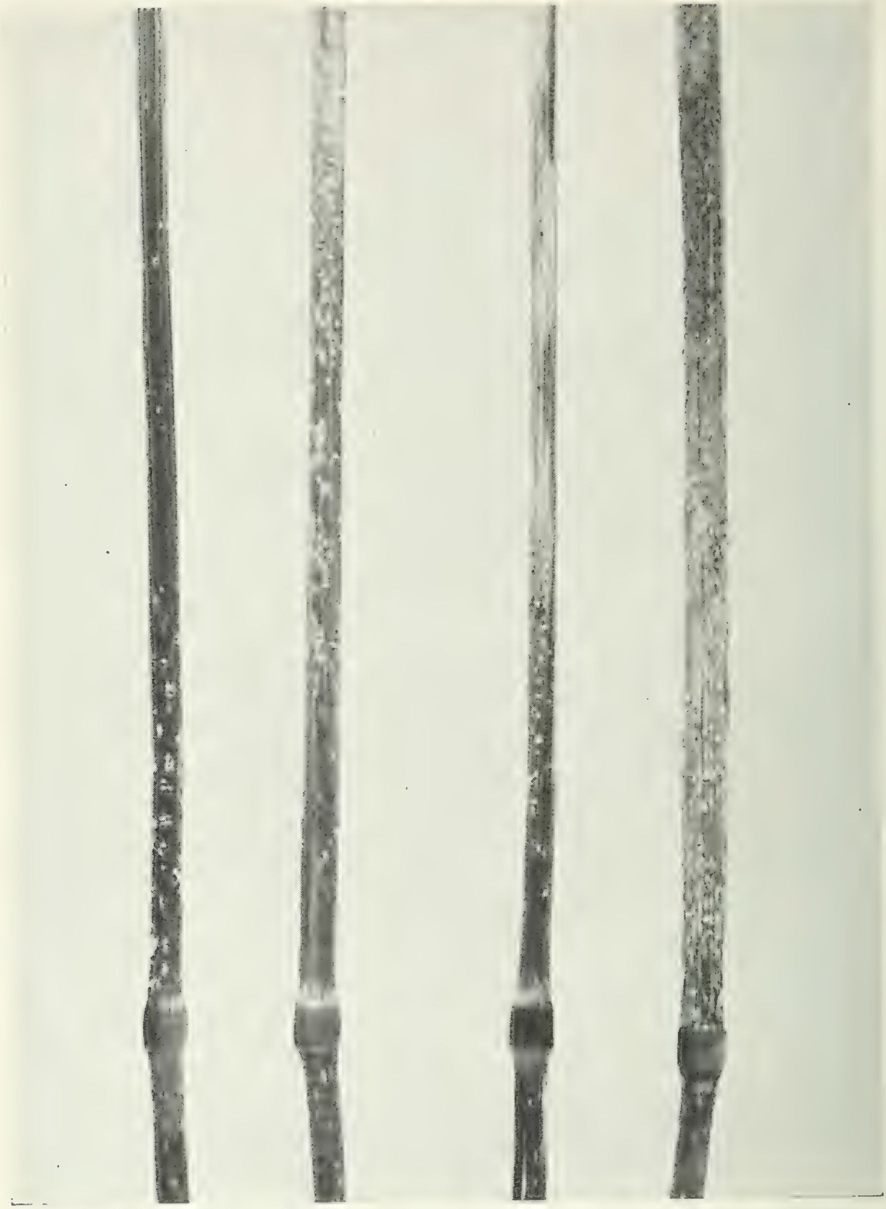

FIGURE 20. UREDINIAL SORI OF PUCCINIA PHLEI-PRATENSIS ON CULMS OF TIMOTHY

Puccinia phlei-pratensis. The same authors the next year, however, establish *Puccinia graminis phlei-pratensis* comb. nov. (1917), and yet they use the name *Puccinia phlei-pratensis* in many places in the same paper. In being thus inconsistent, they admit that the relation of the rust to *Puccinia graminis* is still probably debatable, but they favor including it as a biologic form of *Puccinia graminis* (1917:482).

Arthur, in the *North American Flora* lists the fungus as *Diaceoma poculiforme* (Jacq.) Arth. (*Puccinia graminis* Pers.), but in his new book on plant rusts (1929:328) he calls it *Puccinia graminis phlei-pratensis.* Stakman (1922:251) reverts to Eriksson's original name in summing up the diseases of cereal and forage crops in the United States in 1921, for there he lists the timothy rust organism as *Puccinia phlei-pratensis* Eriks. & Henn.

It is obvious from this brief review of the literature that the name of this fungus has been changed about considerably by taxonomists of the rusts. In view of the following facts, the author favors retaining the name *Puccinia phlei-pratensis:* (1) the fungus has produced neither pycnia nor aecia under controlled conditions; (2) the uredinial mycelium is appreciably smaller than that of *Puccinia gramin s;* (3) teliospores are rare; (4) the fungus overwinters in the uredinial condition in northern latitudes; (5) no evidence of biologic specialization has been obtained; (6) the uredinia are distinctively linear instead of oval or elliptical; (7) the urediniospores are predominantly pyriform rather than oval, elliptical, or elongate; (8) the author who made it a biologic form of *Puccinia graminis* subsequently calls it *Puccinia phlei-pratensis.*

Pathogenicity. Certainly the array of inoculations already cited under the discussion of the suscepts leaves no doubt of the pathogenicity of *P. phlei-pratensis.*

Only one paper seems to exist reporting inoculations with collections of the rust from different places to see if any specialization occurs within the species. Barker and Hayes (1924:369–370) inoculated various clonal lines with collections of timothy rust from different parts of the United States and Canada. " No definite evidence of biologic specialization of *Puccinia graminis phleipratensis* was found." In view of the rather recent work of Craigie (1927) this may be due to the absence of an aecial suscept.

Life history. That the fungus overwinters as perennial mycelium is hardly to be doubted. Eriksson and Henning (1894:141) first advanced evidence in support of this hypothesis. Johnson (1911) developed uredinial sori from overwintered green leaves and he also determined that viable urediniospores may be found after they have passed the winter. Hungerford (1914:337) gives supporting evidence for this opinion.

More than twenty-five separate experiments by the writer on the toxicity of chemicals in which many thousands of spores were examined have revealed some interesting characteristics of the process of spore germination.

The range of conditions suitable for germination seems narrow in many respects. Urediniospores refuse to germinate well if they are scraped from the sori, whereas, if they are washed from the leaf with a stream of distilled water from a wash bottle, germination is good. This may be due to the fact that the latter method removes mostly the more mature spores and does not injure them (Doran, 1922).

The urediniospores either have a specific gravity of less than unity or they are not easily wettable, for they float on the surface of the water drops. When the drops are placed in van Tiegham cells, the spores collect in the lower part of the drop, and then their tubes intermingle so freely that the percentage of germination is almost impossible of determination. If, on the other hand, raised drops are placed on slides in moist chambers, the spores float down to the periphery and appear as a ring of tangled tubes and spores after germination has occurred. Spores that happen to be immersed in the drops do not germinate very well. This indicates a high oxygen requirement.

The best results have been obtained by placing the drops of spore suspension on clean glass slides, and, after the spores have germinated, removing the surplus water with a pipette. The surface tension tends to separate the ring of spores at the edge of the drop so that the individuals are more readily discerned.

Difficulty has been experienced also in allowing too long a time to elapse before counting. After twenty-four hours the tubes become hyaline at the point of emergence from the spore and possess an index of refraction essentially the same as that of water. For this reason, counts must be made as soon as possible after the last spore has germinated. Usually this is within three hours at room temperature. Thus it will be seen that an incredibly short length of time is necessary for complete germination. Another advantage which accrues from making the counts early is that the tangling of tubes is at a minimum.

Even when all these conditions have been complied with, germination of the checks is sometimes still low. A few of the reasons for this are: (1) imperfectly cleaned slides, (2) staling products in the moist chambers, (3) toxic tap water (distilled water should be used), (4) parasitized spores, and (5) immature or overripe spores.

No satisfactory time curve has been obtained, but certainly it must be relatively steep since the peak is reached within three hours.

Temperature has a minimum effect upon germination if sufficient time be allowed. Germination is a slower process at low temperatures. A glance at the graph in figure 21 shows, however, that germination after eighteen hours is just as good at 3° C. as it is at 26° C. Unfortunately a time curve for finding the minimum time for each point was not made on the same day for each temperature chamber. Had that been done, and

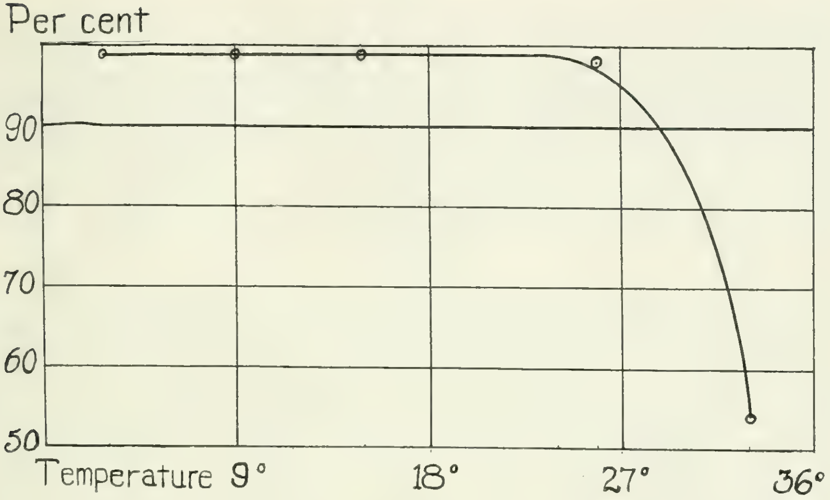

FIGURE 21. EFFECT OF TEMPERATURE ON GERMINATION OF UREDINIOSPORES OF PUCCINIA PHLEI-PRATENSIS

Eighteen hours allows as good germination at 3° as at 26° C.

had a curve been plotted of the germination in each chamber when the maximum germination was attained in the best one, a more exact idea of the effect of temperature could be obtained. It is significant, nevertheless, that complete germination occurred even at 3° C. within eighteen hours. Apparently 33° C. is too high for the best germination.

Evans (1907:446-448) studied the disease cytologically and found that a fine, delicate germ tube runs along the leaf surface and swells into a thin appressorium at a stoma. The protoplasm then passes into the substomatal vesicle and the appressorium dries up.

Evans (1907:447) says also that the substomatal vesicle "gives rise at one end to one infecting hypha only, from which the others subsequently arise." The first septum is formed about the third day when a haustorium begins to develop from the swollen hyphal tip as a little tube which penetrates the cell wall and expands within the cell itself. As the body of the haustorium increases in size, the proximal swollen portion becomes empty and the nuclei pass into the distal end. Sori appear within eight to twelve days.

Urediniospores produced in the primary cycles initiate the secondary cycles which follow each other at approximately fortnightly intervals. Teliospores may or may not develop later in the season (Eriksson and Henning, 1894:140).

Epiphytology

Some epiphytological factor as yet unknown must govern the appearance of timothy rust during the season, since it appears most plentifully as a rule only late in the summer. During 1926, 1927, and 1928 it has occurred but little before the first cutting of hay is made, but it is found frequently on the growth following the first cutting. Kern (1910:417) found the first timothy rust collected in Indiana in a low, wet spot on rich soil. As is the case with wheat rust, the urediniospores are undoubtedly transported by the wind.

Control

A large number of laboratory experiments with various dusts were made to determine what would be likely to serve best in the field as protectants.

Effect of sulfur. In more than ten different tests Bank's colloidal sulfur undiluted always inhibited germination completely except on one slide where about 8 per cent of the spores did show the initial germination. The stock Kolodust always allowed a small amount of germination, but the tubes were dwarfed and distorted considerably, so that infection probably would not have been produced by them had this been on plants instead of glass slides.

A typical data sheet for the three sulfurs is given in table 25.

TABLE 25. EFFECT OF SULFUR DUSTS ON UREDINIOSPORE GERMINATION OF PUCCINIA PHLEI-PRATENSIS

Treatment	Total number of spores	Per cent of germination
Check	453	97.8
Bank's colloidal sulfur dust	521	0.0
Kolodust	483	5.6
300-mesh dusting sulfur	497	16.9

Many other tests showed similar results for the sulfurs[4]. From these data one would be led to conclude that diseases probably can be controlled in the field with sulfur dust.

In the spring of 1927 the effect of sulfur was tried in the field upon timothy left in a two-years-old field after the clover had been killed out. Eight contiguous plots, 1 by 3 rods in size, separated by alleys 1 yard wide, were dusted checkerboard fashion with Kolodust, making four dusted and four undusted plots in the experiment. The first application was made on May 3, just as the first new green leaves were beginning to appear. Subsequent applications were made on May 13, May 29, and June 19.

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[4] The germ tubes produced in contact with various dusts are drawn with a camera lucida in figure 22. Note that the germ tubes which develop in the sulfur dusts are gnarled and distorted, so that they probably are too weak to get through the stomata.

An artificial epiphytotic was started June 4 by surrounding each plot with ten pots of severely rusted plants from the greenhouse. The epiphytotic was fairly well established on the check plots by the time the timothy was harvested on July 11. The rust on the dusted plots appeared less abundant to the eye at the time. The hay was cut just as most of the plants passed the blooming stage. Green weights of the plots are given in table 26.

TABLE 26. RESULTS OF FIELD DUSTING FOR TIMOTHY RUST

(Green weight)

Dusted, 167 pounds	Check, 145 pounds
Check, 177 pounds	Dusted, 202 pounds
Dusted, 188 pounds	Check, 177 pounds
Check, 178 pounds	Dusted, 206 pounds

Observation of the field and of the data as well, reveals that the land increases in fertility towards the south (foot of table 26). The only adequate method of calculating the data therefore is by the pairing of contiguous east-west plots, which gives a mean difference of 21.5 ± 2.47 which is highly significant. This difference shows a gain of 11.8 per cent by the use of the sulfur dust. These figures agree with the laboratory results. Unfortunately, a lack of adequate facilities for further field work precluded a continuation of these studies in 1928.

Effect of copper. In the spring of 1927 a great many laboratory tests of copper-lime dust diluted in lime were made. Dusts containing the following percentages of metallic copper were used: 6.8, 3.4, 1.7, and 0.34. The data obtained are to be found in table 27.

TABLE 27. EFFECT OF COPPER-LIME DUST ON UREDINIOSPORE GERMINATION OF PUCCINIA PHLEI-PRATENSIS

Per cent of copper	Number of tests	Number of spores	Per cent of germination
None + (check)	9	2,209	88.3 ± 2.48
6.8	15	3,969	9.5 ± 1.76
3.4	18	4,292	17.3 ± 2.52
1.7	24	6,211	17.8 ± 1.25
0.34	19	4,691	17.85 ± 2.25

It is obvious that timothy-rust urediniospores are very resistant to copper because they germinated approximately 10 per cent even in the presence of 6.8 per cent of copper. Another fact that the data emphasize is that there is no essential difference in germination whether the spores were in contact with one dilution of dust or the other. Except possibly for the 6.8-per-cent dilution, the copper content appears to have nothing to do with the results. Since the dusts were composed of monohydrated copper sulfate and hydrated lime, the lime was the only substance that could be acting to equalize the dusts, because the copper content was variable. The lime content varied somewhat, too, but since the least amount was about 93 per cent, its relative fluctuation was not great. This conclusion at the time of the tests was not without foundation because several tests with lime alone showed marked reduction in germination.

A subsequent experiment seemed to clinch the argument. In this test duplicate slides on which approximately 600 spores were counted for each treatment, 7.2-per-cent copper-lime dust and lime alone, showed an average of 16.3 per cent germination in the copper lime and 19.3 per cent in the lime alone. A reference to the drawings in figure 22 shows that germ tubes growing in the presence of copper-lime dust appear normal. It would be of interest to find an inert carrier for the monohydrated copper sulfate which would not of itself affect the spores, in order to determine just how toxic copper really is. Bentonite alone in one experiment reduced the germination approximately one-third. Incidentally, in a single experiment the spores germinated 16.6 per cent in 4–4–50 bordeaux mixture also.

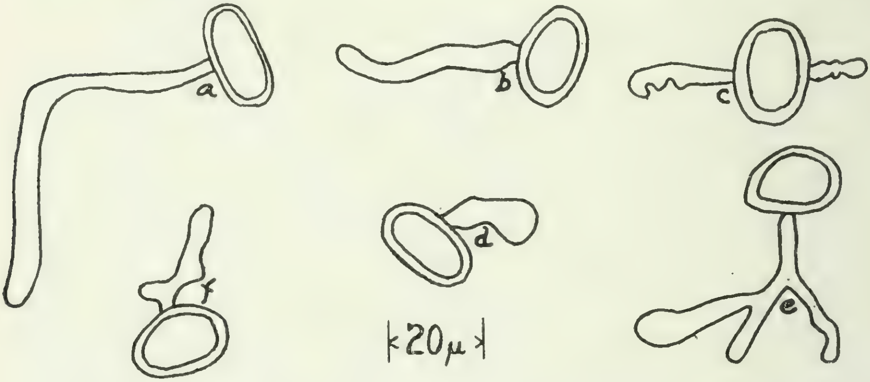

FIGURE 22. GERMINATING UREDINIOSPORES OF PUCCINIA PHLEI-PRATENSIS, SHOWING THE TOXIC EFFECTS OF FUNGICIDES

a, Check; *b*, in copper-lime dust; *c*, *d*, and *e*, in 200-mesh dusting sulfur; *f*, in Bank's colloidal sulfur dust

The copper sulfate might be diluted in water to determine the minimum point of toxicity.

No studies with copper-lime dust in the field have been made, but the evidence in hand indicates rather clearly that sulfur dust at least may be depended upon to control timothy rust in the field and that copper-lime dust if not altogether inefficient, would certainly be less satisfactory than the sulfur.

Investigators have maintained that the only hope of combating the trouble successfully lies in the development of disease-resistant plants. Timothy varieties do vary in their susceptibility to the disease, as indicated under varietal susceptibility, and, since no specialization of the fungus seems to occur, the development of disease-resistant varieties of timothy is not such an unpromising method as it seems to be in the breeding of resistant varieties of wheat.

Barker and Hayes (1924:370) show that resistance or susceptibility seems to depend upon a single factor pair. "Resistance appears to be dominant, and a close approximation to a 3:1 ratio was obtained in the progeny of self-fertilized resistan plants." Proytchoff (1928) confirms the conclusions of Barker and Hayes in general, but he thinks that he has some evidence of modifying factors influencing the degree of resistance. The nature and the number of these factors were not determined.

CERCOSPORA LEAF SPOT OF GRASSES

Suscepts

According to the writer's collections, Cercospora leaf spot attacks timothy, orchard grass, red top, blue-grass, and *Alopecurus geniculatus* in New York. In published records the following additional species of grasses are recorded as suscepts: *Bromus unioloides*, *Hordeum jubatum* (Spegazzini, 1911:436), *Bromus marginatus*, *Bromus sitchensis*, *Hordeum nodosum*, *Elymus glaucus*, *Elymus robustus* (Johnson and Hungerford, 1917: 69), *Alopecurus fulvus*, *Oryza clandestina*, *Milium effusum*, *Poa nemoralis*, *Glyceria fluitans*, *Glyceria plicata* (Schroeter, 1908:498), *Anthoxanthum odoratum*, *Arrhenatherum elatius*, *Avena elatior*, *Avena sativa*, *Brachypodium* sp., *Cynosurus cristatus*, *Glyceria aquatilis*, *Poa compressa*, *Poa trivialis*, *Secale cereale* (Lindau, 1907:794), and *Avena bulbosa* (Tracy and Earle, 1895 b:115).

The disease

The malady, for which the name *Cercospora leaf spot* is proposed, probably is the most important disease of timothy in the State at the present time. It is serious also on orchard grass. It seems to have been recorded first in America by Trelease (1887). The writings indicate that it is very widely distributed, at least in America and Europe.

Symptomatology

Probably the most characteristic symptom is circular to elliptical, small, purple-brown spots, with brown to gray centers, dispersed over the leaf surface. These spots seem to be more in evidence when the disease occurs on mature tissues, for they are most frequently conspicuous late in the season. These spots on timothy leaves may be seen in figure 23. These leaves show chiefly, however, the long aniline to sulphine yellow streaks (Ridgway, 1912) which result from elongation of the spots parallel to the midrib. An incipient stage of the latter type of spotting is shown at *A* in figure 23.

The disease appears to be slightly different early in the season on orchard grass as the following notes on symptoms, taken in May, 1928, indicate: The spots occur on leaf sheaths and blades where they tend to congregate on the midrib, resulting in a cracking of the tissues at that point. They appear at first as small elongate, hydrotic areas, but they enlarge rapidly, soon becoming one to two centimeters long or even longer. The hydrotic center early in the morning when the dew is on is deep olive gray (Ridgway, 1912), but upon drying it becomes deep dull gray. This is surrounded by a band of pecan brown, and this in turn by a narrow strip of maize yellow. The spots occurring toward the proximal end of the leaf cause it to turn yellow over the entire length.

All the symptoms described were noted on the spots that bore the minute black conidial stromata. These are arranged in rows in the center of the spots parallel to the veins of the leaf.

Etiology

Name, history, and classification of the pathogene. Fuckel in 1863 described the causal organism as *Scolecotrichum graminis* in *Fungi Rhenani Exsiccati* (1863:134). Subsequently many species of Scolecotrichum as well as varieties of *S. graminis* were described on grasses. Recently, however, von Höhnel (1924:6–7) has shown that the genus Scolecotrichum probably is invalid. He places this fungus in *Passalora graminis* (Fckl.) Höhn. and includes *S. compressa* Allescher (Hedwigia 35^2:34. 1896) and *S. graminis* (Fckl.) var. *nana* Sacc. (Annales Mycologia 3:515. 1905.) as synonyms, saying that they pertain to the young condition of the fungus.

Von Höhnel seems to have overlooked *Cercospora graminicola* (Tracy and Earle, 1895 a:179) in his disposition of the fungus. Type material of this fungus, which was kindly lent to the writer by Dr. Charles Chupp, was compared with various American collections of *S. graminis* and specimens in European exsiccati for which the writer is indebted to Dr. John A. Stevenson of the Office of Mycology, Bureau of Plant Industry, Washington, D. C. The identity of the two fungi is obvious. The genus

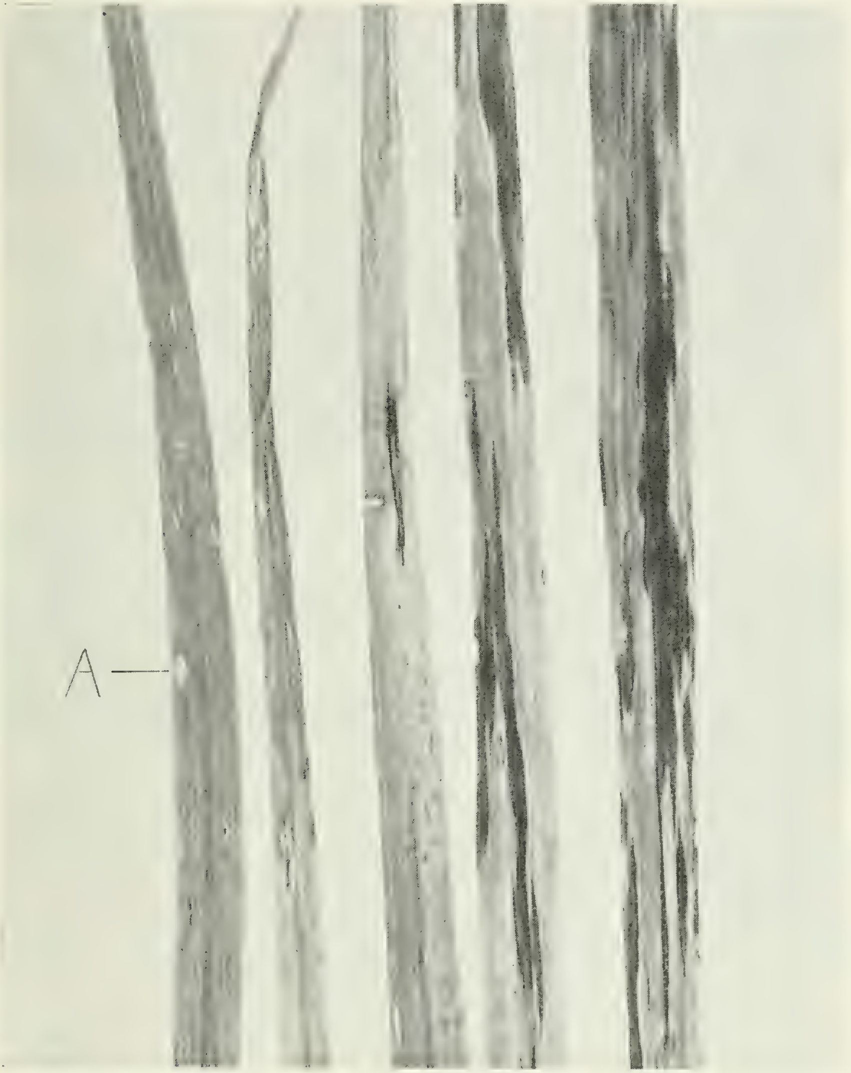

Figure 23. LESIONS CAUSED BY CERCOSPORA GRAMINIS ON LEAVES OF TIMOTHY
Long yellow streaks result from the elongation of spots (A) parallel to the midrib

Cercospora seems a more logical place for the fungus than the genus Passalora, because the latter has many-septate conidiophores, while these are chiefly continuous or rarely septate. In the absence of type material of *S. graminis*, of course, no disposition can be considered as final: nevertheless the fungus can be included without reasonable doubt as *Cercospora graminis* (Fckl.) comb. nov. The following names are listed as synonyms with the authority for considering them as such inclosed in parenthesis after each:

Scolecotrichum graminis Fckl. (writer, 1929)
Passalora dactylina Pass (Lindau, 1907:794)
Passalora graminis (Fckl.) Höh. (writer, 1929)
Scolecotrichum graminis var. *nana* Sacc. (Von Höhnel, 1924:6)
Scolecotrichum compressum Allesch. (Von Höhnel, 1924:7)
Cercospora graminicola Tracy & Earle (writer, 1929)

Species that are closely related to if not identical with *Cercospora graminis* are *Scolecotrichum graminis* var. *avenae* (Eriksson, 1891:28); *S. sticticum*[5] (Berk. and Br.) (Oudemans, 1874:315); *S. graminis* (Fckl.) var. *brachypoda*[6] (Spegazzini, 1911:436); *Passalora punctiformis* and *P. hordei* (Otth, 1868).

The identity of these species cannot well be determined in the absence of the type material. The similarity of their descriptions evidently indicates that further investigation might result in their incorporation as synonyms of *Cercospora graminis*. *Azozma punctum* Lacr. has sometimes been included as a synonym, but von Höhnel (1924:6) states that this is an error.

Fuckel (1869:107) listed *Scolecotrichum graminis* as the conidial stage of *Sphaeria recutita* Fries (1822:524), although as Schroeter said (1908: 498), "aber wohl ohne genügenden Grund." Von Höhnel (1924:6) referred this fungus to *Carlia recutita* (Fr.) Höh.

Single spore cultures of *C. graminis* produce what are undoubtedly perithecial primordia on sterilized wheat grains, but no method of treatment has yet induced these to form asci and spores. Exposing the cultures to ultra-violet light and to outside conditions that were successful for *Pseudoplea trifolii* was of no avail in this case. In the spring of 1928 perithecia of a fungus provisionally identified at the time as *Mycosphaerella* sp. were found on overwintered leaves of orchard grass bearing conidial fructifications of *C. graminis*. The ascospores were biseriate, hyaline, and one-septate with one cell frequently somewhat larger than the other. Ten spores measured 7.2 to 9.0 μ by 23.0 to 32.4 μ in size, thus corresponding with conidia of *C. graminis*. An attempt was made to get a pure culture rom these spores, but they failed to shoot upward to agar. This fungus may be the same as that described by Fries, but his description is meager. *Leptosphaeria culmorum* Awd., which occurs on various grasses, such as quack grass, timothy, and orchard grass, is probably not the perfect stage of *C. graminis*, since single ascospore cultures from these three grasses are in no wise comparable with single spore cultures of *C. graminis*.

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[5] Oudemans' figure is typical of *C. graminis* and the spores measure 40μ long according to Lindau (1907: 794).

[6] The description of this fungus might well pass for that of *C. graminis*.

No information is available on the pathogenicity of the fungus, its life cycle, or measures for its control.

HELMINTHOSPORIUM LEAF SPOT OF BLUE-GRASS

Suscepts

Kentucky blue-grass suffers from Helminthosporium leaf spot in the field, and orchard grass has been inoculated successfully in the greenhouse with a spore suspension. Four cereals, wheat, barley, rye, and oats, were inoculated in the greenhouse. No infection occurred, although blue-grass inoculated at the same time did become infected.

The disease

History and range

It appears that in New York blue-grass is almost universally affected with this disease, since specimens have been collected in practically every planting examined during 1926, 1927, and 1928. Drechsler (1923:687) has collected it at many stations on Long Island, as well as in Maine, Massachusetts, Connecticut, Virginia, Maryland, Illinois, and Wisconsin. The disease was first mentioned by Drechsler (1922:35). Monteith (1925) apparently is the only other author who makes any reference to this malady.

Economic importance

The economic importance of blue-grass lies in its use on lawns or golf courses as well as in pastures and meadows. Since this disease rarely interferes with the beauty of the grass to the casual observer, it probably is of minor importance in spite of its ubiquity. The disease does remain, however, as an incipient menace to blue-grass, because it was observed to make one lawn very unsightly under moist conditions.

Symptomatology

The disease occurs on leaf blades and sheaths. The first symptom to appear following inoculation is a minute hydrotic spot which soon enlarges and assumes a characteristic oval shape, at first uniformly reddish or brownish in color. Later a grayish center is differentiated. Lesions vary in size from almost invisible points up to 10 to 15 millimeters in length and 1 to 3 millimeters wide, depending upon the width of the leaf.

Although in the field the spots vary considerably in color, still the general aspect is one of a light-colored center surrounded by a margin of reddish, purplish, or brownish hue. Comparison with Ridgway's color standards (1912) give: for the center, hazel, pale ochraceous-salmon, light grayish vinaceous-lilac, pale vinaceous-lilac, brownish drab, and pale brownish drab; for the margin, Sanford's brown, Prout's brown, yellow ochre, vinaceous-brown, taupe brown, anthracene purple, blackish brown (1), and dull purplish black. Typical foliar lesions are shown in figure 24.

Infections in the greenhouse do not appear to show as dark shades as in the field, especially on the margin. Here colors such as eugenia red van Dyke red, vinaceous-brown, or Indian lake prevail. Seedlings growing in inoculated soil in the greenhouse have been observed to damp off at the ground line after developing the characteristic reddish lesions.

On leaf sheaths the spots are not so regular as on the blades. Large, irregular, brown blotches usually obtain here, so that the greatest damage probably occurs on the sheaths at the bases of the leaves.

Etiology

Name, history, and classification of the pathogene. Helminthosporium vagans Drechs., the causal agent in this disease, was discovered by Charles Drechsler and was first reported in 1922 (1922:35). A year later (1923) he described the fungus as a new species. Drechsler states that the fungus resembles *Helminthosporium sativum*, P. B. K., the cause of foot rot of wheat, in having thick-walled, dark-olivaceous spores, but it differs from the latter in having larger conidiophores, in growing slower, and in sporulating only meagerly on potato dextrose agar. *H. sativum* spores, when they germinate, produce germ tubes only from the terminal cells, but in *H. vagans* they arise from the central segments as well.

Pathogenicity. The writer isolated the pathogene, but no spores were found in cultures on sterilized wheat kernels, oat agar, potato agar, or solidified synthetic media of Coons (1916) and Leonian (1924).

The pathogenicity of the fungus was demonstrated in two trials by planting seed in soil contaminated artificially with a pure culture. The organism was reisolated from the reddish brown lesions produced on seedlings which frequently damped-off. The fungus was reisolated moreover from infections that were obtained by using mycelium covered with moist cotton as inoculum.

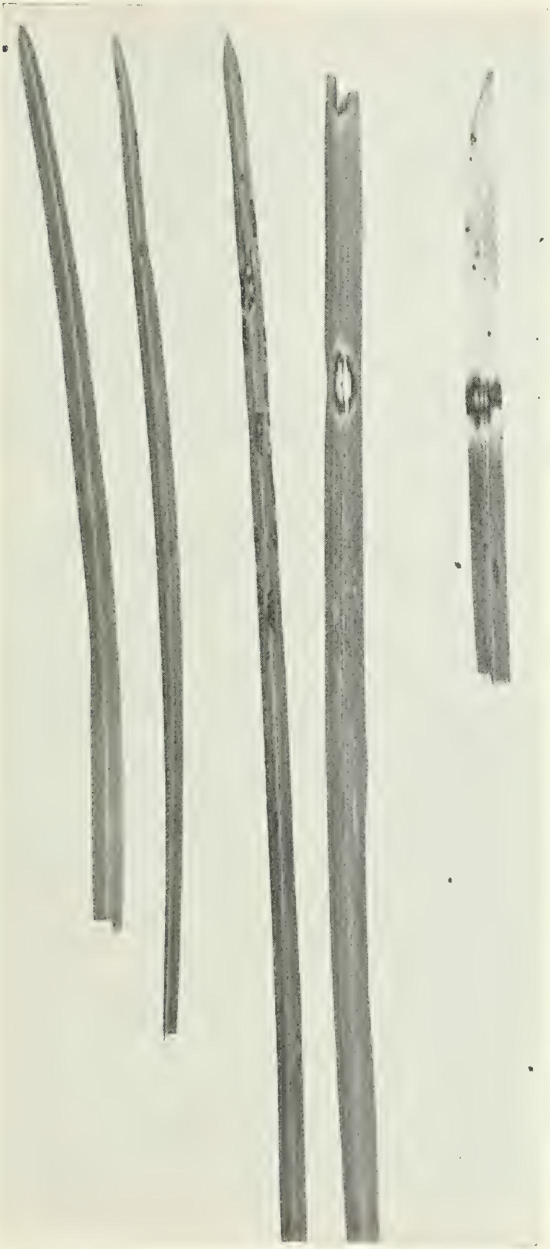

FIGURE 24. LESIONS CAUSED BY HELMINTHOSPORIUM VAGANS ON LEAVES OF BLUE-GRASS

After extensive investigations a method of obtaining conidial production in culture was perfected.[7] The spores thus produced were used for inoculation trials in which the typical leaf symptoms were produced within nine days on blue-grass. The pathogene was reisolated from these lesions.

Physiologic specialization. Christensen (1922) and Stevens (1922) showed that *Helminthosporium sativum* P. B. K. causing the Helminthosporium disease of cereals was a composite species of several physiologic strains. Christensen in a later paper (1926:12) separated several forms by their reactions on culture media, rate of growth, thermal relations, amount of sporulation, zonation, the readiness with which they mutate, and lastly by their pathogenicity.

A test for the presence of such strains in the species *Helminthosporium vagans* Drechs. was undertaken in a small way by the writer. During the summer of 1927 several isolations of the fungus were made from blue-grass in as many localities in New York State as possible. The culture number, the place of collection, and the date of these isolations, are shown in table 28.

TABLE 28. ISOLATIONS OF HELMINTHOSPORIUM VAGANS

Culture no.	Place of collection	Date of collection
127	Ithaca	June 21, 1926
128	Ithaca	June 21, 1926
231	Elba	July 2, 1927
232	Ithaca	June 22, 1927
242	Horseheads	July 31, 1927
244	Mecklenburg	July 31, 1927
251	Ithaca	August 12, 1927
271	Enfield Glen	August 17, 1927
272	Sennett	September 7, 1927

Single spore cultures of all the strains were made except for number 271 which was made as a tissue planting and never was converted to a single spore culture.

Tests of the reaction of the various strains to four culture media were made. Coons' (1916) synthetic medium with 2-per-cent agar, oatmeal agar,[8] cornmeal agar, and potato agar, were prepared and plates were poured with 20 cubic centimeters of each. For each isolation triplicate plates of each medium were inoculated as uniformly as possible in the center with a piece of agar containing mycelium. All of the plates were placed on a laboratory shelf under identical conditions. The diameter of the thalli to the nearest millimeter was determined daily at the same hour, by making two measurements at right angles to each other. Plates containing occasional contaminations were discarded. Measuring the thalli every day was unsatisfactory because of the marked daily variation under the conditions of the experiment.

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[7] The fact that the fungus was found to sporulate by placing transfers on oat or potato agar on a north window ledge according to the method of Jones (1916), encouraged the writer to investigate the stimulus to spore production, the results of which are described briefly. It appears that light is the factor usually limiting spore production in the laboratory where the cultures were usually kept in darkness or semi-darkness. Transfers in quadruplicate to oat agar were made in March, 1928, for all the possible combinations of the following conditions: sealed, unsealed, covered with black paper, uncovered, on north window ledge, and in the greenhouse. Spores were produced in abundance within two weeks only in those tubes that were uncovered whether sealed or not. No spores could be found in those from which light had been excluded. The sealed tubes remained moist, while the unsealed ones dried somewhat, indicating that moisture is unimportant. Ultra-violet light was not a factor, inasmuch as it will not pass through the glass of the test tubes. Direct sunlight is not important, because the tubes on the north window ledge were in diffuse light only. Alternating temperature does not appear to exert much effect, since spores were produced in a subsequent experiment when the transfers were placed in constant temperature incubators heated by incandescent bulbs.

The data are summarized in table 29. These same figures would have been obtained by measuring the thalli at the end of the experiment instead of daily.

TABLE 29. AVERAGE DAILY INCREASE IN DIAMETER (IN MILLIMETERS) OF THALLI OF VARIOUS STRAINS OF HELMINTHOSPORIUM VAGANS

Medium	Culture no.								
	231	232	251	127	272	271	128	242	244
Potato	2.66	2.13	2.11	2.88	2.58	3.21	3.54	13.1	11.67
Oat	5.21	5.25	6.27	4.87	3.48	3.50	5.47	12.0	11.33
Cornmeal	4.39	4.04	5.48	4.54	4.85	3.00	5.65	12.6	11.90
Coons'	....	....	....	2.39	2.95	....	3.30	....	.....

The table indicates that 231, 232, 251, and 127, as well as 271 and 272, may be placed in a group which grow at about the same rate. Isolation 128 might conceivably be placed in this group, but it grows uniformly somewhat faster than do the others. Cultures 242 and 244 are in a class by themselves and differ from each other in that 242 grows slightly the faster.

From observations of color reactions and general appearance made at the conclusion of the experiment, the following cultures were grouped together as probably representing a single physiologic form: 127, 231, 232, 251, 271, and 272. The color of this group by reflected light on potato agar ranges from blackish green gray (Ridgway, 1912) to Sanford brown in the center. There is a narrow apricot-orange margin which is sharply defined. It will be recalled that these colors appear in the symptomatology complex. On oat agar the aerial mycelium is somewhat more prominent in the center, where it ranges from a blackish brown to an olivaceous black. Oat agar appears to be the best differential medium for the forms. On cornmeal agar differences are not striking. The mycelium is much thinner, tending in early stages to be almost invisible within an internal ring about 3 or 4 millimeters from the edge. This gives the effect of a white halo around the young thallus. The mycelium is a dark ivy green on cornmeal agar.

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[8] The media were prepared as follows: Coons', 10 cubic centimeters each of M/5 magnesium sulfate plus 7 H_2O, potassium acid phosphate, asparagin, and maltose, per liter; oat, 35 grams of 3-minute oat flakes and 14 grams of agar per liter; cornmeal, 30 grams of Quaker cornmeal and 14 grams of agar per liter; and potato, 210 grams of peeled potatoes and 18 grams of agar per liter.

Culture 128 stands definitely alone, since it produces an abundance of "white islands," which appear almost like minute somewhat feathery white Clavarias in the plate. These structures, which are arranged in concentric circles, are made up of closely compacted, large, barrel-shaped hyphal cells that branch and fray out slightly at the distal ends. They are from 2 to 4 millimeters high. The submerged mycelium has a velvety olivaceous black appearance which fades to a medal bronz at the edge, fringed with white. After the culture is two or three weeks old, the blackish submerged mycelium tends to grow up and over the white islands, dulling their appearance.

Cultures 242 and 244 also appear greatly different from any of the others and different from each other. Culture 242 is Hiathi gray uniformly over the plate on oat agar. A few small white clumps, quite unlike those of 128, occur scattered over the plates. There is a small, sharply defined central portion of lilac gray which changes to dawn gray on the margin. Cornmeal agar separates out this strain because the mycelium here is a dark grayish olive in the center, grading off to a light olive gray at the edge instead of the usual dark ivy green. As the description indicates, the mycelium of this form is much lighter than of any of the others.

Culture 244 resembles 242 but it is somewhat darker and shows a white fluffy center on all agars. It is dark olive gray around the edge on potato agar, storm gray on oat agar, and deep olive on cornmeal agar. The submerged mycelium is somewhat rhizomorphic on the last medium.

On Coons' synthetic medium the fungus grows very slowly. That medium is useless for differentitation.

After having found these differences in culture, the next logical objective was to determine whether differences in pathogenicity occurred, but a lack of time prevented this from being carried to completion.

Mutation. It seemed desirable also to determine if *Helminthosporium vagans* mutates in culture. No sign of sector mutation was observed in any of the strain work outlined previously until suddenly toward the end a single "white island" similar to those described under culture 128 arose from the otherwise blackish mass in one of the plates of oat agar of culture 271. Upon transferring one of these carefully to another plate of oat agar, as 271 *d*, it developed almost precisely like culture 128 except possibly that the submerged mycelium tended to be more nearly pink. White islands developed profusely. Three subcultures of white islands failed to give increased general whiteness of the culture. A hint of dark color in the submerged mycelium remained.

Culture 271 also produced another sector mutation of a reddish brown color, which was designated 271 *a*. This was distinctly different from the original culture. Subsequently in plate culture the white-island mutation, 271 *d*, produced a definite sector 271 *da*, which corresponded with the original 271. Then the reddish brown mutation, 271 *a*, formed two additional sectors. One of these, 271 *aa*, was definitely pink, and the other reverted to the 271 type. Still later a sector was found that formed heavy rhizomorphic branches on cornmeal agar and was distinctly of a dark yellow green, 271 *ab*. It probably corresponds to the original form. One other mutation from culture 271 arose. It was of a gray-green color resembling very much culture 244. Cultures 272, 232, 231, and 251 were then transferred to oat-agar plates, and the pink form corresponding with 271 *aa* arose from each.

Using mycelium in lieu of spores, these so-called mutations were inoculated into blue-grass to see whether they would retain their characteristics after assuming a parasitic relationship. The most pathogenic mutation was culture 271 *da*, the white-island form. It induced a violent case of the disease within four days. Of less virulence were 271 *aa*, the pink form, 271 *ca*, another white-island form similar to 271 *da*, and 271 *ba* which resembled culture 244.

Reisolation was successful with cultures 271 *da*, 271 *ca*, 271 *ba*, and 271 *aa*. The white islands of the first two, as well as the rapid growing and gray characters of 271 *ba*, came out unchanged, but the pink form of 271 *aa* was not recovered.

It seems that mutation occurs in the species but that many of them are relatively unstable, as evidenced by the reversion in culture. This seems to be especially true of culture 271 *aa*, the pink form. Pink color is more or less evident in lesions in nature, and tends to occur somewhat in all the cultures, especially on oat agar. Pink color also tends to form in the presence of contaminations, so that it is possibly a response to environment. Culture 271 *aa* arose as a direct sector and bred true except for occasional reversions to the original condition. Although in reisolations it was recovered as the original form, this does not prove that the mutation 271 *aa* is not a definite entity, since it might merely have reverted again to the original condition within the suscept.

Reversion did not occur within the suscept in the case of the two white-island mutations, 271 *da* and 271 *ca*, which arose independently. These were reisolated without change. The tendency for white islands to form in test-tube cultures of 231, 232, and 251 was also observed. These have never been seen in culture 127 or culture 272. The white-island condition seems to be as stable as, or more stable than, what is called the *typical condition*.

Culture 271 *ba*, since it was reisolated unchanged, is probably also relatively stable. The chief point of the mutation studies is that, of the four physiologic strains postulated from the cultural studies, three were produced by mutation corresponding to cultures 128 and 244, and the typical condition, as represented by culture 231, was secured as a reversion from a mutation. A strain corresponding with culture 242 was not obtained by mutation, but a new one, 271 *aa*, appeared.

Effect of temperature on cultural characters. As one of the corallaries of the studies of the effect of media, culture 127, representing the typical fungus, was grown in triplicate plates containing 20 cubic centimeters each of Coons' synthetic medium at various temperatures. The diameters of the thalli were measured daily to the nearest millimeter. A duplicate experiment of shorter duration revealed the same results.

Both experiments demonstrate that the best growth occurred at 25° C., with a minimum somewhere below 3° and a maximum between 30° and 35° C.

The data from the first experiment are presented in the graphs shown as figures 25 and 26. In figure 25 is shown the growth curve based on the final diameter of the thalli. The peak is very sharp. The daily growth rate at the various temperatures is plotted in figure 26.

Life history. Although the complete life history of *Helminthosporium vagans* is not known exactly, yet enough data have been accumulated to warrant their inclusion here.

In the spring two primary sources of inoculum exist. To judge from a single year's observations, the fungus lives over winter in lesions on living leaves. Also, mycelium and spores were overwintered in test tubes of oat agar on a north window ledge where the temperature reached as low as 5° to 10° F. on several occasions. In the spring the agar had liquified from freezing, but transfers gave typical cultures of the fungus. The vegetative mycelium is capable therefore of living all winter, at least under the conditions of this experiment, and of growing again in the spring. This indicates the possibility of its living in refuse or in living leaves of blue-grass. No perfect stage has yet been found, although immature perithecia which may belong to this fungus have been observed on overwintered infected leaves. Conidia from dead leaves probably serve as the chief inoculum.

An experiment was made in duplicate to test the effect of temperature on spore germination. Counts after twenty-four hours revealed that the spores had germinated 98 per cent or better in all temperature chambers from 3° to 35° C. Obviously the fungus will germinate over a very wide range of temperature if sufficient time be allowed. It is interesting that germination occurs well at 35° C., whereas no appreciable growth occurs when plate cultures are incubated at that temperature. At 9° and at 3° C. there is a hint of shortening of the tubes, but that is a retardation of growth rather than a reduction of germination.

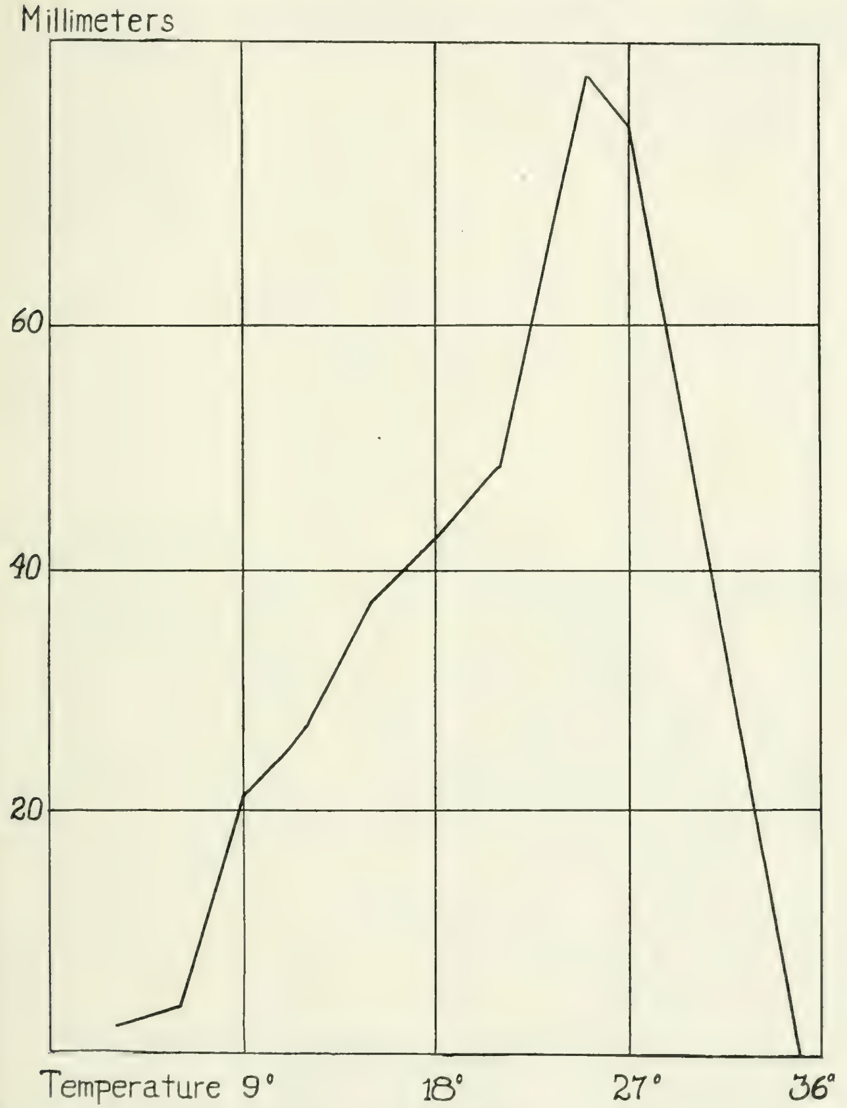

FIGURE 25. EFFECT OF TEMPERATURE ON THE GROWTH OF THALLI OF HELMINTHOSPORIUM VAGANS IN TRIPLICATE PLATES OF 20 CENTIMETERS OF COONS' SOLIDIFIED SYNTHETIC MEDIUM

Diameters of the thalli in millimeters

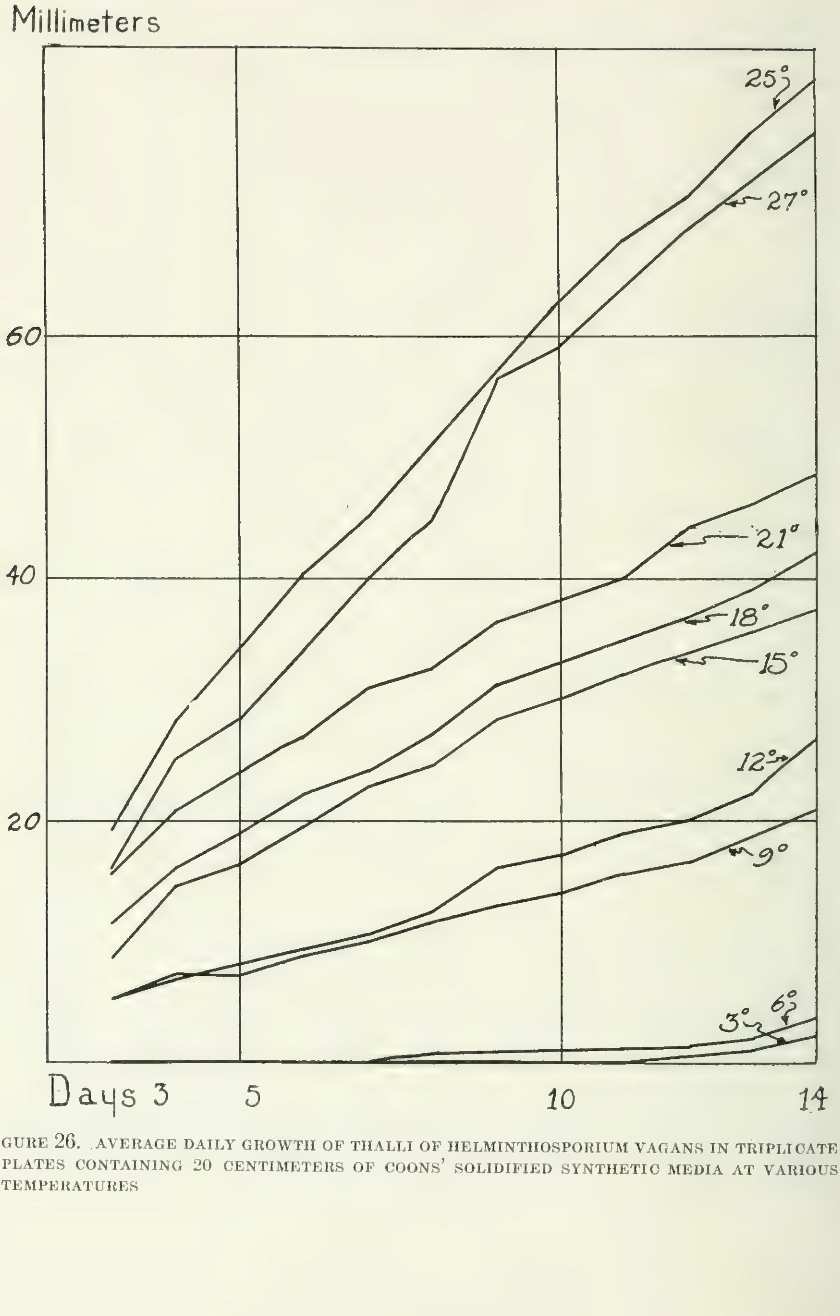

FIGURE 26. AVERAGE DAILY GROWTH OF THALLI OF HELMINTHOSPORIUM VAGANS IN TRIPLICATE PLATES CONTAINING 20 CENTIMETERS OF COONS' SOLIDIFIED SYNTHETIC MEDIA AT VARIOUS TEMPERATURES

The optimum could best be found by determining the minimum time necessary for complete germination at each temperature, but the time consumed probably would not be warranted. Instead, a series of slides with spore suspensions were placed at 25° C. and a time curve was procured. The data are given in table 30 and are presented graphically in figure 27.

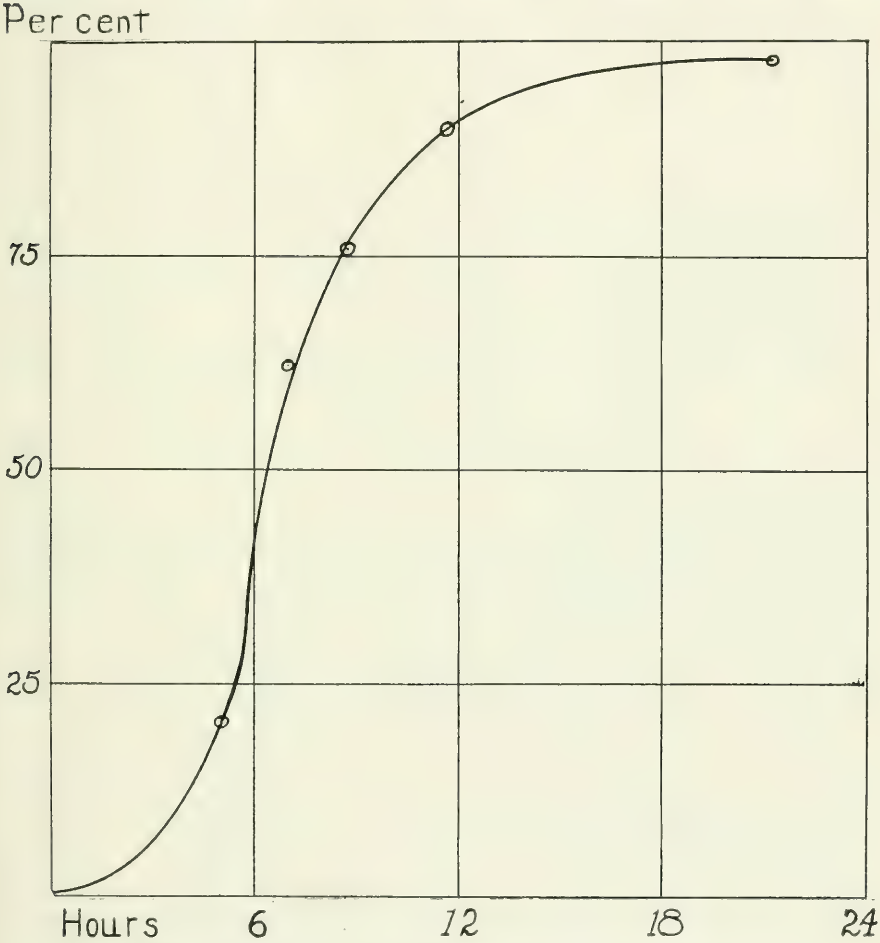

Figure 27. germination of conidia helminthosporium vagans at 25° c.

TABLE 30. Effect of Time on Conidial Germination of Helminthosporium Vagans at 25° C.

Hours elapsed	Total number of spores	Per cent of germination
5	391	20.5
7	388	58.7
8¾	407	75.7
11¾	426	90.2
21¼	359	97.4

The first visible hydrotic symptom may be observed within four days in the greenhouse. At the end of a week following inoculation, the typical symptoms are well developed.

Since spores are found in greatest abundance on severely diseased or dead material, the fungus probably pursues a distinct saprogenic existence, although little is known about it.

Many secondary cycles may occur in one season, because only from one to two weeks need elapse between the generations of spores.

Control

All the studies on control have been directed toward testing copper- and sulfur-dust protectants.

The effect of sulfur upon the germination of the spores of the pathogene was tested in three experiments and the effect of copper-lime dust in four, each of which was made in duplicate. The data from these experiments are summarized in table 31.

TABLE 31. Summary of the Effect of Copper and Sulpur Dusts on Conidial Germination of Helminthosporium Vagans

Treatment	Total number of spores	Per cent of germination
Check	1,793	97.8
7.2-per-cent copper-lime dust	645	7.7
3.6-per-cent copper-lime dust	1,159	58.8
Bank's colloidal sulfur dust	1,012	89.1
Kolodust	635	99.3
300-mesh dusting sulfur	691	91.1

Even the most toxic form of sulfur, Bank's colloidal sulfur undiluted, had little or no effect in inhibiting germination. Copper-lime dust, however, especially when used at full strength, seems to be promising for field experiments. Even the germ tubes that did emerge are abnormal, gnarled, and stunted, indicating strong toxic effects. In figure 28 a spore germinating in the presence of 7.2-per-cent copper-lime dust is shown. This spore seems to be typical of the effect of copper. Most of the spores that germinated in this dust produced the three-branched germ tubes from the terminal cells and almost never from any of the central segments. The sketch of spore germination in distilled water may well represent the germination in sulfur dusts.

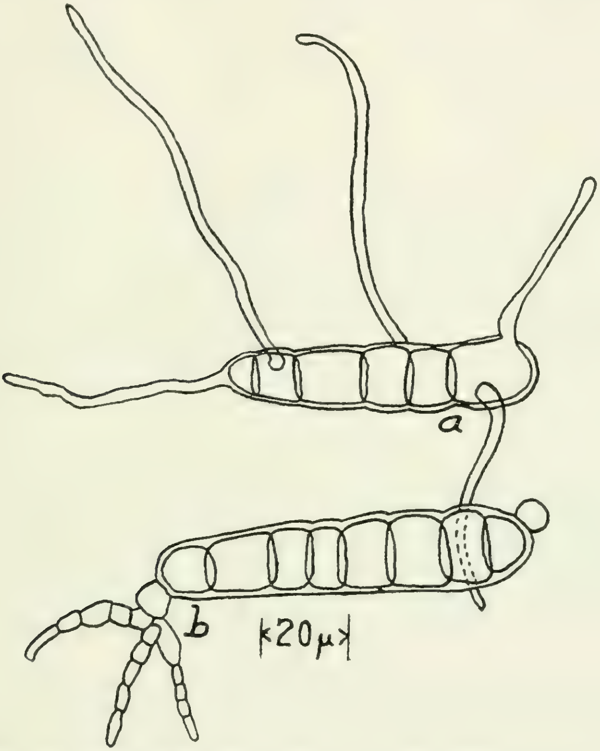

FIGURE 28. GERMINATING CONIDIA OF HELMINTHOSPORIUM VAGANS, SHOWING THE TOXIC EFFECT OF COPPER-LIME DUST

a, Germination in distilled water; *b*, in copper-lime dust

These tests indicate that commercial copper-lime dust applied under moist conditions will probably control the disease satisfactorily by preventing spore germination and penetration, and that any sulfur treatment is likely to be ineffective in the field.

The two diluents, lime and talc, were tried alone to see whether they were exerting any toxic effects. They were used only once, but this was sufficient to demonstrate their non-toxicity, as shown in table 32.

TABLE 32. EFFECT OF DILUENTS ON CONIDIAL GERMINATION OF HELMINTHOSPORIUM VAGANS

Treatment	Total number of spores	Per cent of germination
Check	422	99.2
Lime	429	98.0
Talc	434	99.0

HELMINTHOSPORIUM LEAF SPOT OF ORCHARD GRASS AND RED TOP

Helminthosporium leaf spot on red top appears first as small spots of Dresden brown (Ridgway, 1912) surrounded by a salmon-colored zone which fades into the normal green of the leaf. The spots are small at first, but they rapidly elongate in the direction of the long axis of the leaf, forming streaks which finally result in the death of the affected organ. The fungus fructifies abundantly on old lesions, appearing as a black fuzz. The development of the malady on orchard grass is less well known, but the fungus was isolated once from yellowed areas on leaves of this suscept.

The causal agent was identified provisionally as *Helminthosporium triseptatum* Drechsler (Drechsler, 1923:686), since the spores were almost without exception two- or three-septate. Drechsler believes that it is a saprophyte. Spores have never been seen in culture on Leonian's medium and they are rare on oat agar and potato agar, but they are produced abundantly on sterilized wheat kernals at room temperature. The mycelium is black and otherwise characteristic of Helminthosporia in culture.

Conidia thus produced in cultures isolated from orchard grass were germinated at the following temperatures: 3°, 9°, 15°, 21°, 25°, 30°, 35° C. in duplicate tests. The percentage of germination ranged from 98.6 at 3° C. to 99.5 at 35° C.

These counts which were made after forty-eight hours indicate that temperature within this range really has little effect upon germination if time enough be allowed. It is interesting, however, that the fungus germinated well at 35° C., while the spores of most of the other fungi investigated were injured at this temperature.

The results of two fungicide experiments are given in table 33.

Copper-lime dust appears promising for the control of this disease. The average high germination in 3.6-per-cent copper-lime dust diluted in lime is of interest, but standard 20 80 copper-lime dust probably would have inhibited germination completely.

TABLE 33. EFFECT OF SULFUR AND COPPER-LIME DUSTS UPON CONIDIAL GERMINATION OF HELMINTHOSPORIUM TRISEPTATUM

Treatment	Number of tests	Number of spores	Per cent of germination
Check	4	840	98.9
300-mesh dusting sulfur	4	869	98.2
Kolodust	4	855	97.5
Bank's colloidal sulfur dust	4	882	96.5
3.6-per-cent copper-lime dust	4	907	33.8
Lime alone	2	412	99.7
Talc alone	2	432	99.2

TIMOTHY SMUT

Scant attention in this investigation was devoted to timothy smut because of the excellent discussion of the disease by Osner (1917). An attempt was made to culture the smut, using the method of Christensen and Stakman (1926:981) by which they immersed corn-smut spores in 1-per-cent copper-sulfate solution and removed them at intervals up to seventy-two hours and made streak plantings with them on potato agar. No success crowned this effort probably because a period of after-ripening is necessary for the germination of these spores, as pointed out by Davis (1924 b). Davis (1928) has published a note on physiologic specialization of the fungus.

SILVER TOP

Sporotrichum poae Pk., described originally by Stewart and Hodgkiss (1908), causes what they call *silver top* on blue-grass, orchard grass, and timothy. The disease is rather common in June as the grasses are heading. It frequently accompanies the killing of the heads, which stand out prominently among the green ones. Several attempts to culture the fungus failed. Stewart, however, did culture it.

POWDERY MILDEW

Powdery mildew of grasses is common, but it seems to play a minor rôle in the meadow-crop economy in this State. Sulfur will keep it easily in check. To discuss in detail the enormous amount of work of Reed, Salmon, and others on specialization of the fungus is outside the scope of this paper.

PIRICULARIA LEAF SPOT OF FOXTAIL

Piricularia grisea, the cause of piricularia leaf spot of foxtail, (*Setaria lutescens* (Wieg.) (Hub.), is a common and a destructive disease in the State. The spots, watersoaked at first, are unmistakable. Later there is an irregular margin on old spots of raw umber color (Ridgway, 1912) and a center of light neutral gray to pallid purplish gray. In some cases the spots occur on leaf sheaths.

OTHER DISEASES

Professor F. C. Stewart of the New York State Agricultural Experiment Station at Geneva, New York, gave the writer a note regarding Rosen's (1924) bacterial disease of foxtail, which he had found near Geneva in 1919. He sent material to Rosen, who confirmed the diagnosis but remarked that the spots are different from those usually found in Arkansas in being larger and more regular in outline and somewhat more conspicuous in color. The head smut of Setaria caused by *Ustilago neglecta* Niessl. is common.

The discussion of diseases of other grasses found incidentally in meadows would occupy too much space in this paper in proportion to their importance. Miss Sampson (Stapledon and others, 1922) has discussed the diseases of miscellaneous grasses to a limited degree as they occur in Wales. Although the writer has been unable to obtain the publication in the Cornell library, there appears to be an extensive discussion of grass diseases by Bunting (1928) as they occur in the Gold Coast of Africa.

DISCUSSION AND SUMMARY

This investigation was undertaken because of the lack of easily accessible information concerning diseases of meadow crops in the State, crops which have a greater total valuation than that of any other single crop in the State.

The chief contributions of this paper are: (1) the development of a relatively simple technic for estimating loss in the field from leaf spots of meadow crops; (2) estimates of losses induced by these leaf spots in New York in 1928; (3) the symptomatological characterization of the various diseases; (4) a review of the nomenclatorial tangles to determine the most logical name for certain of the pathogenes; (5) observations and experiments upon life histories; and (6) provisional determination of the possibilities for field control of the diseases by dusting.

When an investigator sets himself to work on the diseases of a crop, he is confronted immediately with the desirability of knowing which are the most important diseases and what the losses are that are caused by each. To ascertain the losses from stem and foliage diseases is difficult but is of peculiar significance where the foliage is the most valuable portion of the plant, as is the case with most meadow crops. The method for determining losses presented herein, and which is based upon an " index of infection " procured from a careful survey of almost 200 fields in the State, gives some measure of the absolute loss in addition to rating the diseases according to their relative economic importance. The method, however, probably gives too conservative an estimate of destructiveness because it does not measure the subsequent effects which defoliation produces. With this method, however, a measure of loss in individual fields based upon the percentage of diseased leaves may be obtained.

Since it is not always easy for those who are only indirectly concerned with meadow-crop diseases to distinguish the various maladies in the field, a rather full discussion of symptoms is given for each.

The names of meadow-crop pathogenes in many instances are sadly in need of revision. A careful mycological study has been made of those considered in this paper, in order to clarify this situation, but the correct name for some of them still remains unsettled.

Many observations and experiments upon the factors governing spore germination have been made in connection with the work on several of the pathogenes, which throws new light on this phase of their life history.

It is a common assumption that the control of meadow-crop diseases by the application of fungicides is impractical and visionary. Interestingly enough, however, almost no one has tried heretofore to control these diseases with fungicides to see whether the idea really is impractical. Inasmuch as the majority of New York farmers of necessity must own a machine for the application of fungicides to such crops as potatoes or fruit, they should use that machine as much as possible in order to reduce overhead expense. Since almost all, if not all, the farmers grow some hay crop, usually timothy, clover, or alfalfa, they should find the control of the disease of these crops profitable. It may be agrued that the machine would destroy more of the crop than the increase from the applications of fungicides, but it is a well-known fact, which the writer verified during field experimentation, that meadow crops possess a remarkable power of recovery from being " trailed down " when they are young and growing vigorously. This ability to recover diminishes as the plants grow older. Wherever field tests with dusts demonstrate the feasibility of control by judicious applications early in the season, injury from driving through the crop will be of little importance.

Dusts only have been considered during the course of these investigations because of practical difficulties in the application of liquid sprays. The advantages of dusting that are of peculiar importance in this connection are: (1) the smaller weight to be transported; (2) the greater speed with which the operation is accomplished; (3) the lightness of the dust enabling it to filter down through the thick mat of leaves, giving better coverage; and (4) the less time and labor required. Of course, copper-lime dust must be applied when the leaves are wet, but, since the growth of meadow crops is more dense than most others, the leaves remain wet longer. Sulfur dust has been tested more intensively in this work than has copper-lime dust because of its greater adaptability for field work.

A grand summary of all tests made with fungicides appears in table 34. Since there are two tests in each experiment, the number of separate experiments for any particular treatment is just half the number of tests. An odd number of tests indicates that one duplicate was lost.

Other points of a more general nature concerning the fungicide tests

TABLE 34. SUMMARY OF THE EFFECT OF DUST FUNGICIDES UPON THE GERMINATION OF SPORES OF FUNGI CAUSING MEADOW-CROP DISEASES

Fungus	Check		Sulfurs						Copper lime				Lime alone		Talc alone	
			200-mesh		Kolodust		Bank's colloidal		7.2-per-cent		3.6-per-cent					
	Number of tests	Per cent of germination	Number of tests	Per cent of germination	Number of tests	Per cent of germination	Number of tests	Per cent of germination	Number of tests	Per cent of germination	Number of tests	Per cent of germination	Number of tests	Per cent of germination	Number of tests	Per cent of germination
Macrosporium sarcinaeforme	42	96.9	12	95.6	14	94.8	12	95.6	14	0.0	14	0.0	14	94.9	13	95.4
Uromyces trifolii hybridi	14	95.9	10	80.2	10	44.8	10	43.3	6	34.1	....	......	4	85.4	....	......
Uromyces trifolii-fallens	20	94.7	11	66.2	10	33.7	17	19.0	8	29.7	....	......	2	97.0	....	......
Cercospora zebrina	4	94.4	2	96.2	....	......	4	51.4	4	0.5	....	......	....	......	....	......
Pseudoplea trifolii	2	95.3	2	98.6	2	95.0	2	94.2	2	0.0	2	86.5	....	......	....	......
Heterosporium phlei	16	91.6	14	26.6	16	9.6	....	......	....	......	....	......	....	......	....	......
Puccinia phleipratensis	18	88.3	2	15.0	2	5.0	10	0.0	15	9.5	18	17.3	2	19.4	....	......
Helminthosporium vagans	7	97.8	6	91.1	4	99.3	6	89.1	6	7.7	8	58.8	2	97.7	2	99.0
Helminthosporium triseptatum	4	98.9	4	98.2	4	97.5	4	96.5	....	......	4	33.8	2	99.7	2	99.2

and not easily included in this table are: (1) the results of laboratory tests of fungicides vary with the dosage of material on the slide, especially if the chemical in question is diluted to or near to the point of minimum toxicity; (2) sulfur dust is no more effective at higher temperatures than at lower ones, which indicates that it does not act through volatilization or, if it does, it volatilizes with sufficient rapidity at low temperatures to be effective in the cases recorded; (3) hydrated lime apparently has no fungicidal effect on any of the fungi tested except *Puccinia phleipratensis*, but it appears to inhibit markedly the germination of the spores of this fungus, being as toxic as the copper sulfate in copper-lime dust; (4) talc has no visible effect upon spores of *Helminthosporium vagans*, *H. triseptatum*, or *Macrosporium sarcinaeforme;* (5) staling products accumulate in moist chambers used for germinating spores, so that the dishes should be washed carefully after each test.

Most of the essential facts about each disease are summarized in table 35 to avoid a lengthy summary and to facilitate reference.

Some additional facts regarding certain of the pathogenes that do not appear in table 35 may be stated briefly: (1) spore production in culture, induced in *Helminthosporium vagans* and *Pseudoplea trifolii*, by exposure to out-of-door conditions is due to the effect of light; (2) culture mutations in *Helminthosporium vagans* are stable as a rule after passage through the suscept, although work with fungous mutations reported prior to the time this work was initiated had not taken this factor into account; (3) spores of *Macrosporium sarcinaeforme* germinate equally well at all temperatures from 3° to 35° C.; (4) no cicatrice is laid down in red-clover leaves to halt the invasion of this pathogene; (5) powdery mildew has been shown to be very deleterious to red clover, contrary to most opinions; (6) by removing the water from the leaves, the fungus causes them to be deficient in photosynthetic ability, which results in stunting, the most significant symptom of powdery mildew; (7) clover extract stimulates the germination of the conidia of *Erysiphe polygoni* and *Dothidella trifolii;* (8) cool nights and warm days appear to be conducive to the spread of *E. polygoni;* (9) the pathogene causing the Cercospora leaf spot of clovers and alfalfa is *Cercospora zebrina* Pass.; (10) the writer agrees with Petrak that *Pseudoplea trifolii* is the cause of similar diseases of the clovers and alfalfa, and that *Stagonospora meliloti* also causes diseases that are similar to each other on the same suscepts; (11) girdle of alfalfa, sweet, and red clovers, and bacterial leaf spot of clovers, have been found in New York; (12) *Cercospora graminis* is the name that should be applied to the fungus commonly called *Scolecotrichum graminis;* (13) *Helminthosporium vagans* attacks orchard grass as well as blue-grass (producing mutations which pass unchanged through the suscept), consists of physiologic forms, has been induced to sporulate in culture by exposure to light, and overwinters as mycelium in living leaves.

TABLE 35. SUMMARY OF ESSENTIAL POINTS ABOUT THE MEADOW-CROP DISEASES DESCRIBED IN THIS PAPER

Disease	Name of pathogene	Plants affected	Chief symptom	Loss in the State	Fungicide sensitivity
Macrosporium leaf spot	*Macrosporium sarcinaeforme*	*Trifolium hybridum, T. pratense, T. repens, Medicago sativa, Melilotus alba*	Concentrically ringed brown spots	1.36 per cent	Copper
Alsike clover rust	*Uromyces trifolii-hybridi*	*Trifolium hybridum*	Minute, powdery, cinnamon-brown pustules	0.81 per cent	Sulfur
Red clover rust	*Uromyces trifolii-fallens*	*Trifolium pratense*	Minute, powdery, cinnamon-brown pustules	0.09 per cent	Sulfur
White clover rust	*Uromyces trifolii trifolii-repentis*	*Trifolium repens*	Minute, powdery, cinnamon-brown pustules	0.84 per cent	Sulfur
Pseudopeziza leaf spot	*Pseudopeziza trifolii*	*Trifolium alpestre, T. arvense, T. cyathiferum, T. fragiferum, T. hybridum, T. incarnatum, T. macrodon, T. medium, T. nigrescens, T. pallescens, T. pallidum, T. pratense, T. repens, T. resupinatum, T. spadiceum*	Small, purple to brown spots, with dentritic borders; small dried apothecium in center	4.62 per cent on red clover	Copper (probably). Sulfur seems valueless
Powdery mildew	*Erysiphe polygoni*	356 species of plants, including *Medicago falcata, M. lupulina, M. sativa; Melilotus alba, M. officinalis; Trifolium agrarium, T. alpestre, T. arvense, T. filiforme, T. hybridum, T. incarnatum, T. involucratum, T. longipes, T. lupinaster, T. medium, T. minus T. monanthum, T. montanum, T. moranthum T. pauciflorum, T. pratense, T. procumbens, T. repens, T. rubens;* 12 species of Vicia, 2 of Phaseolus, 1 of Pisum	Whitish mold on leaf surface	From one-fourth to one-third of the crop in certain cases	Sulfur
Cercospora leaf spot	*Cercospora zebrina*	*Trifolium agrarium, T. alpestre, T. hybridum, T. incarnatum, T. rubrum; Medicago arabica, M. denticulata, M. hispida, M. sativa; Melilotus alba, M. officinalis*	Linear, reddish to smoky spots, usually limited by veins, especially on species of Trifolium	1.27 per cent on alsike clover 1.08 per cent on white clover 0.09 per cent on red clover	Copper
Sooty blotch	*Dothidella trifolii*	*Trifolium agrarium, T. alpestre, T. alpinum, T. elegans, T. filiforme, T. fragiferum, T. hybridum, T. lupinaster, T. medium, T. montanum, T. pratense, T. rubens, T. wormskioldii, T. reflexum, T. repens*	Black, mealy blotches	0.76 per cent on white clover 0.24 per cent on alsike clover	Not known. Sulfur seems valueless
Pseudoplea leaf spot	*Pseudoplea trifolii*	*Medicago falcata, M. hispida, M. lupulina, M. maculata, M. sativa, Trifolium hybridum, T. pratense, T. repens*	Leaves look peppered, spots usually minute	5.59 per cent on white clover 0.48 per cent on alsike clover 0.24 per cent on red clover	Copper

Clover mosaic	Undetermined	*Trifolium arvense, T. hybridum, T. incarnatum, T. pratense, T. repens, T. resupinatum; Medicago falcata, M. lupulina, M. sativa; Phaseolus vulgarus; Vicia faba*	Leaves mottled and wrinkled	Not severe	....
Kabatiella anthracnose	*Kabatiella caulivora*	*Trifolium hybridum, T. incarnatum, T. medium, T. pratense, T. repens, T. suaveolens; Medicago lupulina; Onobrychis sativa*	Elongate, sunken, black lesions on stems. Leaves drooping and dead	Very severe	Not sensitive to sulfur
Stagonospora leaf spot	*Stagonospora meliloti*	*Trifolium hybridum, T. pratense, T. repens; Melilotus alba; Medicago lupulina, M. sativa*	Oval to circular spots on leaves. Spots: light-brown, brittle centers; dark-brown margins	1.08 per cent on white clover	Not known
Girdle	Undetermined	*Trifolium pratense, Medicago sativa, Melilotus alba*	Gray-green, sunken girdles on stems	Rare	....
Bacterial leaf spot	*Bacterium trifoliorum*	*Trifolium hybridum, T. medium, T. pannonicum, T. pratense, T. repens.*	Inky black, angular spots on leaves	Rare	Not known
Fusarium root rots	*Fusarium* spp.	*Trifolium pratense*	Dry brown rot	Not uncommon	....
Downy mildew	*Peronospora trifoliorum*	*Medicago sativa, Trifolium pratense*	White mycelium on water-soaked leaf spot	Rare	Probably copper
Dodder	Cuscuta sp.	*Trifolium* spp.	Yellow threads on **plants**	Rare	....
Rhizoctonosis	Rhizoctonia sp.	*Trifolium pratense*	Soft rot of stems	Rare	....
Sclerotinia stem canker	*Sclerotinia trifoliorum*	*Trifolium pratense*	Black sclerotia on stems	Rare	....
Thielavia root rot	*Thielavia basicola*	*Trifolium pratense*	Roots blackened	Rare	....
Black stem	Undetermined	*Medicago sativa, Melilotus alba, Trifolium pratense*	Elongate black lesions	Very common	....
Heterosporium leaf spot of timothy	*Heterosporium phlei*	*Phleum pratense*	Small, oval spots, mauve-colored center, dark purple margin	Important	Sulfur
Timothy rust	*Puccinia phlei-pratensis*	*Avena elatior, A. fatua, A. sativa; Bromus tectorum; Dactylis glomerata; Elymus virginicus; Festuca elatior; Hordeum jubatum, H. vulgare; Lolium italicum, L. perenne; Poa compressa; Secale cereale*	Powdery, yellowish pustules on culms and leaves	Important on second cutting	Sulfur
Cercospora leaf spot of grasses	*Cercospora graminis*	*Agrostis alba; Alopecurus fulvus, A. geniculatus; Anthoxanthum odoratum; Arrhenathrum elatius; Avena bulbosa, A. elatior, A. sativa; Brachypodium sp.; Bromus marginatus, B. sitchensis, B. unioloides; Cynosurus cristatus; Dactylis glomerata; Elymus glaucus, E. robustus; Glyceria aquatilis, G. fluitans, G. plicata; Hordeum jubatum, H. nodosum; Milium effusum; Oryza clandestina; Phleum pratense; Poa compressa, P. nemoralis, P. pratensis, P. trivialis; Secale cereale*	Aniline to sulphine yellow streaks on blades; hydrotic at first	Most important of timothy diseases	Not determined
Helminthosporium leaf spot of blue-grass	*Helminthosporium vagans*	*Dactylis glomerata, Poa pratensis*	Oval spots, with drab center and reddish brown margin	Most important disease of blue-grass	Copper

TABLE 35 (*concluded*)

Disease	Name of pathogene	Plants affected	Chief symptom	Loss in the State	Fungicide sensitivity
Helminthosporium leaf spot of red top and orchard grass	*Helminthosporium triseptatum*	*Agrostis alba*, *Dactylis glomerata*	Terminals of leaves turn dry and brown	Not common	Copper
Timothy smut	*Ustilago striaeformis*	*Dactylis glomerata*, *Phleum pratense*, *Poa pratensis*, and others	Narrow, linear, black streaks on leaves and leaf sheaths	Unimportant	Not determined
Silver top	*Sporotrichum poae*	*Dactylis glomerata*, *Phleum pratense*, *Poa pratense*	Inflorescence turns white and blasts	Common in June on blue-grass	Not determined
Powdery mildew	*Erysiphe graminis*	Most grasses and all cereals	White mycelium on leaf surface	Common	Sulfur
Piricularia leaf spot of foxtail	*Piricularia grisea*	*Setaria lutescens*	Gray-centered spots with dark margin	Common	Not determined

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STUDIES ON THE FIRE-BLIGHT ORGANISM, BACILLUS AMYLOVORUS

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STUDIES ON THE FIRE-BLIGHT ORGANISM, BACILLUS AMYLOVORUS[1]

A. L. PIERSTORFF

Investigators giving attention to the fire-blight disease of pear and apple during the past several years have again placed in question certain of the concepts which were generally regarded as established. Besides presenting many important facts, these workers have added to the long series of interesting questions which are urgently in need of further study. The writer began a study of certain phases of the fire-blight problem in 1923, and continued at intervals until September, 1926. No attempt is here made to review the voluminous literature relating to fire blight, except as it bears directly on the phases treated in this paper.

PLANTS AFFECTED

Many workers have contributed to our knowledge of the species of plants that may be infected by *Bacillus amylovorus* (*Erwinia amylovora*). For the literature on fire blight preceding the classical work of Burrill (1878, 1881 a, 1881 b), who discovered the causal organism and proved apple, pear, and quince to be suscepts, the reader is referred to the excellent reviews given by Arthur (1887c), Stewart (1913), and Snow (1922). Burrill (1881 b) included lombardy poplar, aspen, maple, elm, linden, ash, lilac (leaves), butternut, and some herbaceous plants (peony), in the list of suscepts. All of the above-mentioned plants have since been eliminated as suscepts. Arthur (1885 b:1183) reports that "unsuccessful inoculation was made upon the mountain ash *(Pyrus aucuparia)*, but as the tissues were already solid when done, and as branches suffering with the disease have since been found, there is no doubt that it can be communicated if the inoculation be properly performed."[2] Waite (1896) includes mountain ash among the species which should be inspected for blight in eradication work. Edwards (1907), in speaking of fire blight, states that the cut-leaved mountain

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[1] Also presented to the Faculty of the Graduate School of Cornell University, June, 1929, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

AUTHOR'S ACKNOWLEDGMENTS. The investigations discussed in this paper were undertaken at the suggestion of Dr. H. E. Thomas. To him and to other members of the staff of the Department of Plant Pathology at Cornell University the writer is greatly indebted for helpful suggestions and criticisms, and he acknowledges his sincere appreciation.

[2] *Sorbus aucuparia* L. For botanical and horticultural names, the *Manual of Cultivated Plants*, by L. H. Bailey, is used as authority.

ash (*Sorbus aucuparia* var. *laciniata* Beissn.) is a menace to the fruit grower. Snow (1922) does not include mountain ash among the list of proved suscepts, and the writer has been unable to find in the literature any reports of inoculations or cultural work which would substantiate the common belief that this plant is susceptible.

Arthur (1885 a: 362) proved by inoculation experiments that the fire-blight organism may attack June berry (*Amelanchier canadensis* (L.) Medic.), English hawthorn (*Crataegus oxyacantha* L.), and an evergreen thorn (*Pyracantha coccinea* Roem.), and later (1887) b) he added wild crab (*Pyrus coronaria* L.). Craig (1897) reported *Crataegus oxyacantha* as a host for fire blight at Ottawa. Later, Edwards (1907), in Ontario, reported that on *C. oxyacantha* var. *splendens* Schneid. the disease is common. Waters (1921) isolated the causal organism from English hawthorn in New Zealand, and added medlar (*Mespilus germanica* L.) to the list. Reed (1914) says that blight was particularly severe on *Crataegus crus-galli* L. in Missouri in 1913. Jones and Morse (1903) proved that the Cheney plum (*Prunus nigra* Ait.[3]) is attacked by blight. Jackson (1915) added the Italian prune (*Prunus domestica* L.). Snow (1922) gave cultural evidence that *Prunus triloba* Lindl.[4] is attacked by *B. amylovorus*. The organism isolated was pathogenic to green pear fruits immediately after isolation, but plum shoots inoculated with it failed to exhibit symptoms of disease. Waite (1907) reported that "the evergreen *Eriobotrys Japonica* is attacked by this disease very commonly in Florida and Georgia, and recently has been found affected in California." Speaking of the Christmas berry, *Heteromeles arbutifolia* Roem., he stated: "In March, 1906, I found a striking specimen, badly attacked, at Vacaville. It had been affected on the blossoms the summer before and several of the twigs contained the living bacilli, they having been carried over the winter. It has since been found at Colusa and several other points in California." Martin (1921) reported successful inoculations on the loquat tree (*Eriobotrya japonica*).

After isolating the organism and making cross inoculations, Paddock (1903: 8) concludes: "The results of these experiments prove that pear blight may attack apricot twigs and fruit and observations show that the disease may do a considerable amount of damage. . . . Blighted twigs were also found on *Prunus simonii* trees which were also thought to be caused by an attack of pear blight." In 1915, Hotson found Royal Ann cherries which were infected by the blight pathogene. Isolation of the causal organism and reinoculation into green cherries caused the fruits to blight. In the same year Hotson reported the yakimine also as affected by blight, and the following year he published a photograph showing a blighted twig. Munn (1918) found that strawberry flowers would blight if they were sprayed with a culture of *B. amylovorus* or if a platinum needle previously dipped into the culture was rubbed over the "nectarial surfaces"; leaves failed to blight when drops of a cultural medium carrying the bacteria were placed on them. Munn states that he has not seen strawberry blight occurring naturally out of doors.

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[3] *P. americana* var. *nigra* Waugh.
[4] *P. triloba* var. *plena* Lindl.

In 1925 Waite reported that he had obtained typical fire-blight symptoms by inoculating the cut surface of a mature Winesap apple. Also, by smearing the cut ends of dormant pear and apple twigs and placing them in water under a bell jar, blight cankers were produced. Semidormant rose cuttings were infected, but with some difficulty.

In the same year, Reimer (1925) reported his extensive tests on the resistance of various species of pear to blight. The most resistant appear to be wild forms of *Pyrus ussuriensis*, an oriental species. *P. communis,* the species on which most of our pear trees are grafted, is the most susceptible. *P. calleryana*, which is highly resistant to blight, is recommended at the present time for Oregon. Reimer has tested all the known species of pear and has found individuals in each species that will become infected by the fire-blight pathogene.

In 1926 Anderson listed *Spiraea* sp. as a suscept for fire blight in Maryland. Two years later, Rosen and Groves (1928) reported that detached twigs of *Spiraea vanhouttei* Zabel, *Chaenomeles lagenaria* Koidz., and rose terminals of the variety Fairfax, were successfully inoculated in the greenhouse with *B. amylovorus*. Natural infection was reported on Burbank plum (*Prunus salicina* Lindl.).

In addition to the above-named hosts, other species have been shown to harbor the blight organism in their floral parts without exhibiting symptoms of disease. Gossard and Walton (1922 : 99) conclude: "These results mean that the blight organism is capable of living in peach, plum and cherry nectar for five days or probably more. . . ." The writer has reexamined some of the reported cases, and as a result of experimental work has added several new species to the list of suscepts.

INOCULATIONS ON HAWTHORN

In the region near Skaneateles, New York, where there are numerous hedges of *Crataegus oxyacantha*, the writer noticed what appeared to be fire blight on many trees in 1923. Isolations were made in the summer of 1924, and these cultures were used to inoculate apple seedlings during the winter of 1925. Infection was readily obtained.

Inoculations were made also in July, 1925, on five water sprouts growing on a large *C. oxyacantha* tree at Ithaca, New York. All five shoots blighted so rapidly with the characteristic symptoms of fire blight that no further inoculations appeared to be necessary. This work confirms the findings of Arthur (1885 a) and of Waters (1921), and, judging from the extended killing of the hedges, one would conclude that *C. oxyacantha* is highly susceptible to *Bacillus amylovorus* in New York.

INOCULATIONS ON STRAWBERRY

(*Fragaria chiloensis* Duchesne, var. *ananassa* Bailey)

Following the work reported by Munn (1918), the writer sought to obtain additional information concerning the pathogenicity of *Bacillus amylovorus* for strawberry blossoms, leaves, crowns, fruits, and runners. Strawberries of the varieties Chesapeake and Premier, and of one unknown variety (hereinafter designated as variety X), were used for all greenhouse tests.

On February 2, 1925, six blossoms of each variety were inoculated in the greenhouse by puncturing with a sterilized needle dipped in a three-days-old bouillon culture. Three days after inoculation, four of the inoculated blossoms, two of Premier and two of the variety X, appeared water-soaked and light brown in color. In eight days these blossoms had turned almost black and were badly shriveled. The pedicels of the blossoms had turned black for a distance of about two inches from the flower. Isolations made from two pedicels about one inch below the blossoms yielded the fire-blight organism, which was checked by inoculation into apple seedlings.

On March 3, 1925, ten blossoms of each of the three varieties were inoculated by atomizing with a bouillon culture of the organism; nine days later three of the Premier blossoms, two of the Chesapeake, and four of the variety X, were dead. In another test, five berries six days old, of the variety X, were inoculated with a needle; six days after inoculation, four of the fruits had been killed by the blight organism. In Plate I, 1, a blighted fruit is shown on the right and a healthy fruit on the left. The writer was unable to produce infection in half-grown or maturer fruits.

On March 9, 1925, one hundred leaves, thirty runners, and the crowns of twenty-four plants, were punctured with a needle dipped in a culture of *B. amylovorus*. Half of the plants were left in the greenhouse, and the other half were placed in a moist chamber in the headhouse for twenty-four hours and subsequently removed to the greenhouse. This experiment was repeated on March 21, but no infection was detected in any of the leaves, runners, or crowns. The cultures used were pathogenic to apple seedlings.

On May 31, 1926, approximately four hundred wild-strawberry blossoms (*Fragaria virginiana* Duchesne) growing out of doors were atomized with a two-days-old culture of the fire-blight organism. Ten days later most of the blossoms had died, from natural causes since very few had set fruit. Two blossoms were found which appeared to have been killed by *B. amylovorus.* Cultures were made from ten blossoms, including the two doubtful ones. One blossom yielded the fire-blight organism. The writer is convinced, however, that very few of the dead blossoms had blighted.

The foregoing experiments substantiate the findings of Munn (1918), and give additional information on the susceptibility of flowers and young green fruits of the strawberry, and on the resistance of leaves, runners, and crowns, to the fire-blight pathogene.

INOCULATIONS ON ROSE

In conversation with Dr. L. M. Massey, of the Department of Plant Pathology at Cornell University, during the spring of 1924, it was suggested to the writer that the rose might be a suscept for fire blight. Dr. Massey stated that he had noticed what appeared to be fire blight on terminals of roses on a number of occasions, but had made no isolations. This prompted the writer to inspect roses rather closely during the growing season, and in the latter part of July, 1924, several blighted terminals of climbing roses of the variety Tausendschön were found in a nursery near Rochester, New York. Isolation from these terminals and reinoculation into apple shoots proved the presence of *Bacillus amylovorus.*

In order to test further the susceptibility of roses to fire blight, cuttings of the following varieties were rooted and grown in the greenhouse: Tausendschön, Climbing American Beauty, White Dorothy, Dr. Van Fleet, Martha Washington, Excelsior, and an escaped sweetbrier of the *Rosa eglanteria* type growing wild about Ithaca. The results obtained by puncturing the terminals with a needle dipped in a seventy-two-hours-old bouillon culture of *B. amylovorus*, are given in table 1:

TABLE 1. Results of Fire-Blight Inoculations on Rose Terminals, February, 1926

	Tausendschön	Climbing American Beauty	Dr. Van Fleet	*Rosa eglanteria*	Excelsior	White Dorothy	Martha Washington
Number inoculated . .	16	18	12	12	8	10	5
Number infected	11	2	0	7	0	0	1

After it was established that the shoots of some varieties of roses could be infected, the question arose as to whether or not blossoms were susceptible. In order to obtain some information on this point, blossoms of *Rosa blanda* Ait., *R. rubiginosa* L., and an open-pollinated seedling of *R. rubifolia* Br. growing on the college farm, were atomized with a forty-eight-hours-old culture of *B. amylovorus* on July 4, 1926, The results recorded on July 23 showed nine of eighty-six inoculated blossoms of *R. blanda* blighted, sixteen of one hundred and seventy-two blossoms of *R. rubiginosa*, and only one, and that a doubtful case, of sixty-three inoculated blossoms of *R. rubifolia.*

On July 20, isolations made from two blighted blossoms of *R. blanda* yielded the blight organism. The infection resulting from needle inoculations on the stems is shown in Plate I, 2.

With the foregoing data as a basis, the writer draws the conclusion that some varieties of roses can be infected by *B. amylovorus.* Waite (1925) states that he obtained infection on semi-dormant rose cuttings. According to the report, no reisolations were made and the cuttings in water under a bell jar were soon overgrown by mold fungi. However, it appears probable that Dr. Waite's conclusion that the cankers were caused by the fire-blight organism is correct.

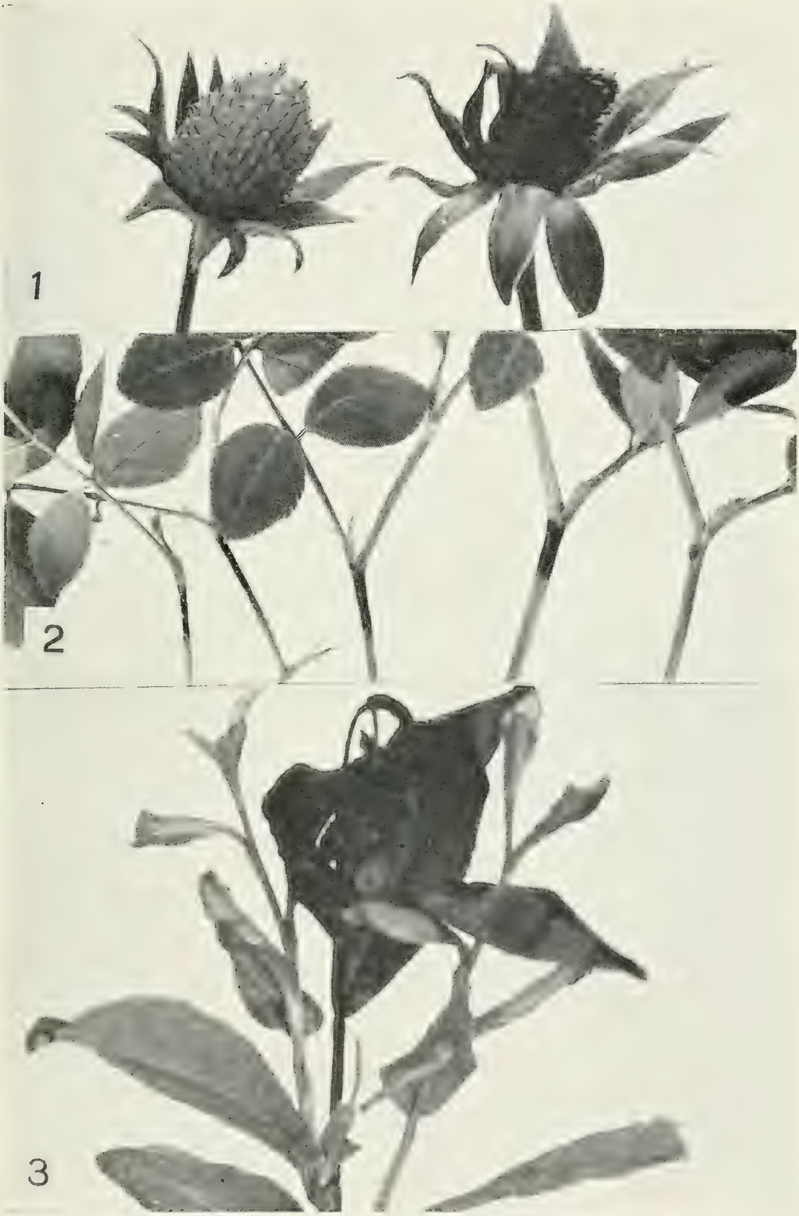
INOCULATIONS ON JAPANESE QUINCE
(*Chaenomeles lagenaria* Koidz.)

In order to test the susceptibility of the blossoms of *Chaenomeles lagenaria* Koidz, to the fire-blight organism, two hundred and fifty blossoms were atomized with a virulent bouillon culture on May 28, 1926. On June 15, all blossoms had fallen and none appeared to have blighted.

On June 22, 1926, twenty terminals of *C. lagenaria* were punctured with a sterile needle dipped in a culture of *Bacillus amylovorus.* On July 4, two terminals were killed back about three inches from the point of inoculation. Ten more terminals were inoculated at this time. On July 21, two of these shoots showed symptoms of blight, and isolation from them and reinoculation into apple seedlings proved the presence of *B. amylovorus.* Three green fruits also were punctured with a needle dipped in a bouillon culture, but frequent inspections revealed no blight symptoms on these. It appears from these results that *C. lagenaria* also is a suscept for the fire-blight disease, but is not likely to be severely affected.

INOCULATIONS ON PHOTINIA
(*Photinia villosa* DC.)

Following a suggestion of Professor H. H. Whetzel, of the Department of Plant Pathology at Cornell University, inoculations were made



RESULTS OF ARTIFICIAL INOCULATION WITH BACILLUS AMYLOVORUS

1, A healthy strawberry fruit and a fruit blighted from artificial inoculation
2, Blight lesions on rose stems of the variety Tausendschön; punctured control at right
3, Twig blight of *Photinia villosa* from artificial inoculation

on terminal shoots of Photinia growing in the nursery at the university farm at Ithaca. On May 28, 1926, twenty-five terminals were punctured with a sterile needle dipped in a bouillon culture of *Bacillus amylovorus*. Inspection on June 14 showed that twenty-one of the twenty-five inoculated terminals had blighted, and some had been killed for a distance of eight to ten inches. Isolation and reinoculation into apple seedlings proved the presence of the fire-blight pathogene. On July 4 the experiment was repeated on ten terminals, of which six blighted. One terminal continued to die back until a limb three-eighths of an inch in diameter had been killed. In Plate I, 3, is shown a blighted terminal in which the organism died and new shoots arose from the live tissue below. The inoculation was made on July 4, 1926, and the photograph was taken on August 3.

SUMMARY OF SUSCEPTS

Either through inoculation and isolation experiments or through identification of the bacteria by means of cultural characters, the following species have been proved suscepts for the fire-blight disease: apple (*Pyrus malus, P. baccata, P. coronaria*), pear (*Pyrus communis* and all other species tested), quince (*Cydonia oblonga*), cherry[5] (*Prunus avium* [Bing and Royal Ann varieties]), plum (*Prunus nigra, P. domestica*, and *P. salicina*), flowering almond (*Prunus triloba*), apricot (*Prunus armeniaca*), hawthorn (*Crataegus oxyacantha, C. oxyacantha* var. *splendens*, and *C. crus-galli*), common firethorn (*Pyracantha coccinea* [*Crataegus pyracantha*]), June berry (*Amelanchier canadensis*), medlar (*Mespilus germanica*), loquat (*Eriobotrya japonica*), strawberry (*Fragaria chiloensis* var. *ananassa* and *F. virginiana*), rose (*Rosa eglanteria, R. blanda*, and varieties Fairfax, Tausendschön, Climbing American Beauty, Martha Washington [probably derived from *R. cathayensis*]), Photinia (*Photinia villosa*), Japanese quince (*Chaenomeles lagenaria*), and spiraea (*Spiraea vanhouttei*). Species which are probably suscepts but concerning which further investigations are needed, are mountain ash (*Sorbus aucuparia* and *S. aucuparia* var. *laciniata*), Christmas berry (*Heteromeles arbutifolia*), yakimine, apricot plum (*Prunus simonii*), the Hortulana plum (*Prunus hortulana*), and additional species of Crataegus.

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[5] On June 17, 1930, a twig blight was observed on sour cherries (*P. cerasus* L.) of the Montmorency variety in several localities in southern Ohio. The symptoms resembled those of fire blight, and cultures yielded an organism which on beef-peptone agar was indistinguishable from *Bacillus amylovorus*. Inoculation into ten vigorous apple shoots produced blight in all of them. The organism was recovered in culture and used a second time to produce infection on apple shoots.

ETIOLOGY

CULTURAL REACTIONS OF BACILLUS AMYLOVORUS

The complete cultural reactions of *Bacillus amylovorus* were not redetermined by the writer, since these have been described by Jones and Morse (1903), D. H. Jones (1909), Stewart (1913), and Smith (1920). These workers have reported the acid tolerance in terms of Fuller's scale. Since the hydrogen-ion concentration is now used by most bacteriologists to express acidity of culture media, the writer has adopted that method, using the quinhydrone cell for making pH determinations.

In order to be certain that growth of the bacteria would not be inhibited in some of the acid cultures to be used in testing for enzymes, Stewart's (1913) work on acid tolerance was repeated. He gives the limit of acid tolerance of *B. amylovorus* as 23 to 25 Fuller's scale. This would be approximately pH 5.90 to 5.70.

Growth in nutrient bouillon, made with prepared beef extract acidified with malic acid, was obtained by the writer as low as pH 4.60, or 36 Fuller's scale. The comparative rate of growth of the bacteria is indicated by the number of colonies in the dilution plates in which a standard volume of culture was diluted (table 2).

TABLE 2. ACID TOLERANCE OF BACILLUS AMYLOVORUS IN NUTRIENT BOUILLON

Original pH		6.27	5.30	4.88	4.80	4.70	4.60	4.53	4.44	4.38	4.02	3.41
pH after eight days at 23° C.	Culture	7.07	6.81	6.10	5.45	5.41	4.68	4.60	4.46	4.41	4.07	3.48
	Check*	6.32	5.38	5.03	4.85	4.80	4.68	4.61	4.48	4.41	4.06	3.48
Number of colonies per square centimeter in third dilution plate		10.9	12.6	7.2	4.6	2.6	0.06	0.02	0	0	0	0

* Checks all sterile.

The tests were made in quadruplicate. At the time when the plates were poured, four agar slants were inoculated from the bouillon cultures with pH of 4.60 and below. After eight days several colonies appeared on the slants inoculated from bouillon at pH 4.60, 4.44, and 4.38, but none from pH 4.02 or below. No growth in the bouillon tubes could be detected with the eye at this range. It is possible that little or no growth took place in these tubes, but that the bacteria originally introduced remained viable. It appears from these tests that *B. amylovorus* is able to grow in a more acid medium than was formerly supposed.

Similar tests were made with Uschinsky's solution, but no growth was obtained below pH 4.70.

Smith (1920:372) states that 15 per cent of the organisms survive exposure to freezing. He does not mention the temperature to which the cultures were exposed. Stewart (1913:337) reports having exposed bouillon and agar cultures to –14° C. for fourteen days, with occasional temperatures as low as –28° C., without killing the organism. Jones and Morse (1903:235) obtained no growth at 0.5° C., and very slow growth at 3° C.

While working on the production of toxins by *B. amylovorus,* the writer had occasion to freeze 25 cubic centimeters of sap, expressed from blighted pear fruits, in liquid air (about –183° C.) for ten minutes. After thawing at 3° C., the sap was tested for viable bacteria and was found to contain them in large numbers. This test was not repeated.

Jones and Morse (1903) gave the upper thermal death point for the bacteria as 43.7° C. (for ten minutes in recently inoculated bouillon). O'Gara (1908) says that all the bacteria are killed on exposure at 55° C. for ten minutes. D. H. Jones (1909) states that the thermal death point in bouillon lies between 45° and 50° C. Stewart (1913:335) says: "The death-point is about 47° C." The writer found, in working with sap of blighted pear fruits, that exposure at 48° C. for ten minutes did not always give sterile cultures. However, 49° C. for ten minutes gave sterile cultures in every case (approximately 60). This slight difference between bouillon and pear-sap cultures might be due to difference in heat conductivity.

From the foregoing evidence it appears that *B. amylovorus* is able to live in an acid medium as low as pH 4.60; to resist extremely low temperatures for a short time in liquid media; and occasionally to survive exposure at 48° C. for ten minutes.

STRAINS OF THE ORGANISM

It is well known from the work of bacteriologists and others that strains of bacterial species exist. Variations in cultures of the fire-blight organism were noticed by Stewart (1913:327-328), who remarks: "It should be noted that a strain from Colorado, No. 7, always made a slower growth than any of the others; while No. 9, recently isolated from apple at Ithaca, New York, was usually the most rapid grower." Stewart uses the term *strain* as indicating different isolations. Jackson (1915) found cultures of *Bacillus amylovorus* from prune to be more virulent than cultures from pear. In New Zealand, Waters (1921) repeatedly isolated from medlar cultures of *B. amylovorus* which showed wavy radiations, in addition to being very virulent. Upon inoculation into pear shoots, ooze was produced in fifty-one hours. Martin (1921) reported the production of a dark green exudate from a loquat tree (*Eriobotrya japonica*) artificially inoculated with a pure culture of *B. amylovorus.* Examination of this ooze showed it to be highly charged with fire-blight bacteria. Reinoculation into other trees failed to reproduce an exudate of the same color, and Marin advances no hypothesis as to the reason for this phenomenal color.

The writer obtained a culture of the fire-blight organism from California, and from its rapid growth on nutrient agar and the production of a light yellow color he was led to think that a mistake might have been made in the sending of the culture or that contamination had occurred en route. However, plating the culture several times in extreme dilutions, staining the organisms with differential stains, and examination under the microscope, failed to show more than one type of organism. The culture was then inoculated into apple seedlings in the greenhouse, and reisolation produced a similar organism. Symptoms characteristic of fire blight occurred on the inoculated apple seedlings.

These and other considerations led the writer to make preliminary tests on the occurrence of strains of *B. amylovorus.* Cultures of the fire-blight organism were obtained as follows:

Culture	Source [6]	Isolated from	Date of isolation
A	Utica, New York	Apple	August, 1924
C	Las Cruces, New Mexico	Pear	December, 1924
Cal	Davis, California	Pear	May (?), 1925
Cl	Davis, California	Apple	December, 1925
D	Ithaca, New York	Apple	July, 1923
E	Horseheads, New York	Pear	July, 1923
F	Ithaca, New York	Apple	July, 1924
M	Grand Rapids, Michigan	Apple	June, 1925
S	Columbia, Missouri	Apple	May, 1925
S C	Clemson College, South Carolina	Pear	June, 1925
T	Knoxville, Tennessee	Apple	May (?), 1925
Ti	Tifton, Georgia	Pear	May, 1926

Inoculation tests were made with these cultures in a planting of several hundred seedlings of Kieffer pear at Ithaca. The variation among these seedlings in habit of growth and in taxonomic characteristics was very noticeable, and was probably greater than exists between many of our varieties of pears. A more detailed description of the variation in these seedlings is given by Thomas (1927–28).

Only trees having six or more vigorous shoots were used. Six different cultures were inoculated into the terminals of each tree, one culture to a terminal. If differences in virulence or adaptation to different climatic conditions were inherent in any culture, by inoculating the cultures on the same trees it was thought such differences would become apparent. At the time of checking the results, the terminal growth killed by blighting was measured. Natural inoculations were few in number during the progress of the tests. It is therefore assumed that when a terminal blighted, the culture inoculated into it was the causal factor. The error resulting from this assumption is thought to be negligible. Before each series of inoculations, all strains used were transferred to bouillon tubes three times, at intervals of forty-eight hours, to insure that actively growing bacteria were present in all cultures. Results of these tests are summarized in table 3. A dash and number—for example, A–1—indicates that the original culture was inoculated into apple and reisolated.

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[6] The writer is indebted to Dr. P. D. Caldis, L. M. Fenner, H. W. Fitch, Dr. L. R. Hesler, and Dr. I. T. Scott, for material from which some of these cultures were made.

TABLE 3. SUMMARY OF RESULTS OF INOCULATIONS ON PEAR SEEDLINGS WITH DIFFERENT CULTURES

Culture	Total number of inoculations	Total number of infections	Per cent infected	Average length of twig blighted
				(Inches)
A–1 or A–2	235	75	31.9	6.1
T or T–1	135	68	50.4	8.6
F or F–1	139	42	30.2	8.3
D	94	8	8.5	8.3
Cal	135	63	46.7	5.6
C	44	1	2.3	4.0
S C	141	66	46.8	7.1
S	145	61	42.1	8.2
Ti	96	50	52.1	5.4
E	96	25	26.0	4.3
Cl	100	28	28.0	6.0
M	50	19	38.0	7.9

It is evident that there is a marked variation in the ability of different cultures to infect seedling pear trees. Culture C should be omitted in any comparisons, as the odds are heavily in favor of the one infection with this culture being accidental. Culture C was indistinguishable from other cultures on artificial media. Cultures T and S infected about six times as many trees as did culture D, although after infection occurred there was not much difference in the length of the twig killed. Culture E was able to infect but half as many trees as did culture T, and when infection occurred it was able to kill but half as much twig growth. It should be noted that cultures D and E were isolated at least twelve months earlier than were any other of the cultures used in these experiments. Culture Cal grew more rapidly and luxuriantly than did any other culture, and it also gave a fairly high percentage of infections; but after infection had taken place, it progressed more slowly and ceased invasions of new tissues earlier than did cultures T, F, D, or S.

Culture A was used as a standard for these tests as well as for the work on enzymes and toxins. In not one of the tests made on five different dates did this culture exceed T, S, or Ti in number of infections or in average of total length of twig killed. Further work is needed to compare the same cultures in a larger number of experiments.

PRODUCTION OF TOXINS

That true bacterial toxins (exotoxins) are formed by *Corynebacterium diphtheriae* (Klebs-Löffler) Lehman and Neuman, *Clostridium tetani* (Nicolaier) Holland, *C. botulinum* (Van Ermengem) Holland, and *Pseudomonas aeruginosa* (Schröter) Migula, is common knowledge among bacteriologists. Antitoxins (the production of which is one of the tests for a true toxin in animal pathology) can be prepared for the organism mentioned. Other groups of bacteria, such as the staphylococci, the streptococci, and the pneumococci, produce endotoxins, which are preformed toxic substances retained in the bodies of the microparasites until released by disintegrative processes. Satisfactory antitoxins have not been prepared for these endotoxins.

Some plant pathologists are of the opinion that bacterial phytopathogenes form toxins under certain conditions, but relatively little study has been devoted to this phase of the subject.

Shortly after the discovery of the causal organism of fire blight by Burrill (1878, 1879), various workers sought to demonstrate a toxic or an enzymic substance produced by *Bacillus amylovorus* as a possible explanation of the killing power of this pathogene. Arthur (1886), with the assistance of a chemist, performed repeated tests for "ptomaines." They concluded that no ptomaines were produced. The methods employed at that early date would probably have destroyed any enzyme or organic toxins produced by the organism. D. H. Jones (1909) made exhaustive cultural studies of the organism, but found no toxin produced in culture. Bachmann (1913) finds that "all of the first changes in the cells which result from infection may be attributed wholly to a loss of water." Stewart (1913:360) heartily disagrees with Bachmann, and points out that if plasmolysis of the host cells is due to water loss, an equilibrium in osmotic pressure should soon be established, the cells should regain their turgidity, and no further killing would ensue. He thought that a toxic or an enzymic substance caused the killing of the host cells, but failed to find such a substance in bouillon cultures. In studying the formation of schizogenous cavities, Nixon

(1927:10) says: "There is first a slight, probably toxic plasmolysis, followed finally by the complete collapse of the protoplast but never its dissolution."

The characteristic color and external appearance of blighted twigs and limbs of apple and pear trees, and the lack of similar symptoms when twigs of these same trees are killed by other organisms, has led some pathologists to think that a toxic or an enzymic substance is present in blighted shoots. Whether this substance is excreted by the causal organism or is a decomposition product, has received little consideration, but the question merits attention. Bachmann (1913:11) suggests this possibility.

The writer, in an effort to demonstrate the presence or absence of a definite toxic substance in blighted fruits, placed green Bartlett pears about one and one-half inches in diameter in large, sterile, moist chambers (after sterilizing with bichloride of mercury). The fruits were inoculated with a forty-eight-hours bouillon culture of *B. amylovorus* by means of a hypodermic needle. After inoculation the fruits were again sterilized with bichloride of mercury for five minutes, and were then rinsed three or four times with sterile distilled water. Fruits treated in this way remained free from contaminating organisms in a large majority of cases.

From ten to fourteen days after inoculation, the fire-blight bacteria had invaded practically the entire fruit, and large drops of milky ooze were exuding from many points and especially from the wounds made during inoculation. The fruits were ground in a food chopper at this time, the parts not invaded by the bacteria being excluded. To a liter of pulp, 100 cubic centimeters of sterile distilled water was added, and the juice was extracted by squeezing through cheesecloth. A portion of the extracted juice was centrifuged for thirty minutes at 2000 revolutions per minute, and was then filtered through filter paper. The remaining portion of juice was filtered through cotton to remove some of the organic matter. Subsequent reactions showed little or no difference in the two methods of filtering.

The juice from healthy green pears was expressed and used as a check, being treated in every respect similarly to that from blighted pears. The juice from both blighted fruits and check (healthy green pears) was placed in test tubes in 10-cubic-centimeter quantities, four tubes being used for each lot, and was treated as follows: to lot 1, a crystal of phenol about the size of half a pea was added; to lot 2, a crystal the size of a whole pea; to lot 3, 0.5 cubic centimeter of chloroform; to lot 4, 1 cubic centimeter of chloroform; to lot 5, 2 cubic centimeters of chloroform; to lot 6, 0.1 cubic centimeter of toluol; to lot 7, 0.2 cubic centimeter of toluol; lot 8 was heated to 48° C. for ten minutes; lot 9, to 49° C. for ten minutes; lot 10, to 48° C. for fifteen minutes; lot 11, to boiling for ten minutes; lot 12 was left untreated.

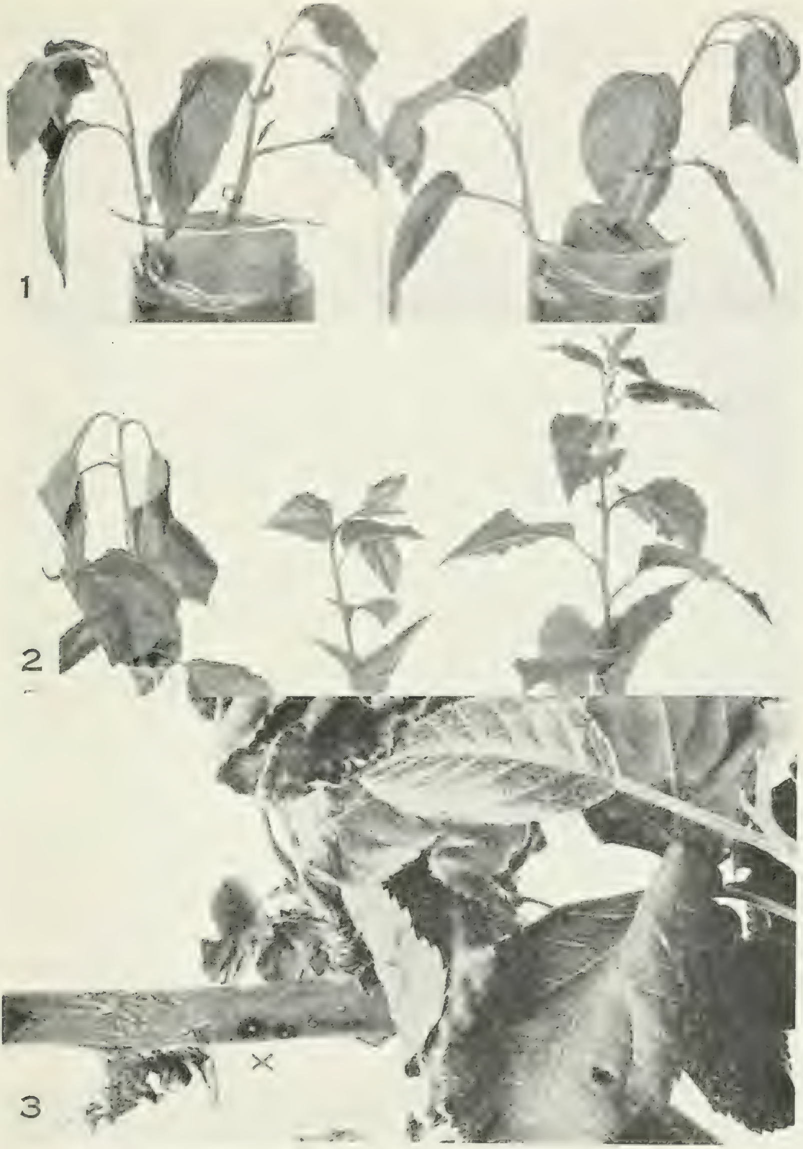
After the various lots had been treated as indicated, cultures were plated from two tubes of each lot to ascertain whether or not the fire-blight bacteria had been killed. It was found that juice from blighted fruit treated with 5 per cent of chloroform and that treated with 1 per cent of toluol did not always give sterile cultures unless it was well shaken after addition of the germicide. This precaution was observed in subsequent tests. One tube from lot 8 was found to contain live bacteria, and in later tests all heated tubes were heated either to 48° C. for fifteen minutes, or to 49° C. for ten minutes.

The freshly cut ends of rapidly growing French seedling crab-apple shoots were inserted in two tubes of each lot, and the results were noted. Thirty minutes after the twigs were inserted, the tips of those in the tubes containing heated juice from the blighted fruit began to droop. In a few minutes, the twigs in the other tubes containing juice from blighted fruit lost some of their turgidity. After forty-five minutes, all the twigs in the juice from blighted fruit, irrespective of subsequent treatment of the juice, showed some wilting. The twigs in the heated juice were drooped as far as the morphological structure of the stem permitted. The checks were all turgid except in those tubes containing phenol, 10 per cent of chloroform, and 20 per cent of chloroform. Equivalent amounts of these germicides in distilled water caused similar drooping after forty-five minutes. Heating the juice from healthy green pears or adding 1 per cent of toluol appeared to have little influence on the apple shoots during a period of two and one-half hours. At the end of this time all the twigs in tubes containing juice from blighted fruit were drooped, while the checks were turgid except in those cases previously noted. The tests were repeated three times with different lots of pears, and comparable results were obtained in each case.

After two hours, some of the wilted twigs were removed from the tubes of juice from blighted fruit, about an inch of stem was cut off the base under water, and the twigs were placed in water to note whether recovery occurred. At the end of two hours the twigs were again turgid. Shoots that had been in the juice from blighted fruit for twenty-two hours did not regain turgidity when they were removed to distilled water.

At the end of fourteen hours, one set of twigs was removed, the solutions were diluted 50 per cent with distilled water, and fresh twigs were inserted. Again the same drooping action was noted, but the time interval was approximately doubled.

It was at once apparent that mechanical clogging might account



APPLE SHOOTS WILTED AFTER BEING INSERTED IN BLIGHTED PEAR JUICE AND BACTERIAL OOZE ON APPLE TWIG

1, Left beaker, center, apple shoot wilted in untreated juice from blighted pears; at side, apple shoot wilted in the diffusate outside of the collodion membrane. Right beaker, arrangement similar, but juice frozen to −183° C. The photographs were taken 1½ hours after the twigs were introduced into the liquid

2, At left, apple twig in juice from blighted pears to which 1 per cent of toluol had been added; at center, apple twig in juice from healthy green pears to which 1 per cent of toluol had been added; at right, apple twig in 1 per cent of toluol in distilled water

3, "Hold-over" canker on apple twig 5/16 inch in diameter; recent oozing is shown at the cross mark

for the wilting of the twigs. In an attempt to determine this point, some juice from blighted fruit that had been heated to 49° C. for ten minutes was dialyzed through parchment membranes by placing the juice inside the cells and distilled water on the outside. After twenty-four hours, the cut ends of twigs were placed in the dialyzate inside the cells and in the diffusate on the outside. The twigs in the juice wilted as before, but those in the diffusate remained turgid.

The question of adsorption by the parchment membrane entered into consideration and collodion membranes were constructed, 50- to 100-cubic-centimeter beakers being used as forms. By duplicating the arrangement of the parchment membranes and permitting the solutions to dialyze for twenty-four hours, it was found that twigs placed in the diffusate outside the collodion cells *wilted as rapidly as did twigs placed in the dialyzate inside the cells* (Plate II, 1).

Similar results were obtained if untreated juice from blighted pears was used inside the collodion cells. Cultures made from the water on the outside of the cells failed to reveal the presence of any viable fire-blight bacteria. Likewise, the diffusate contained no undissolved organic matter capable of clogging the vessels of the apple shoots.

As previously stated, juice from blighted fruit that had not been heated nor treated with germicides was dialyzed. This would invalidate the argument that heating produced toxic substances by the disintegration of organic compounds. To check further on this point and to ascertain whether freezing would kill fire-blight bacteria, a quantity of juice from blighted pears was frozen to –183° C. for ten minutes. After thawing at 3° C. and dialyzing through collodion, this juice produced results similar to those with untreated juice from blighted fruit.

In Plate II, 2, are shown apple shoots after they had been inserted for two and one-half hours in (a) juice from blighted pears (at left), (b) juice from healthy green pears (in middle), and (c) distilled water (at right). To all solutions 1 per cent of toluol had previously been added.

After apple shoots had been inserted in juice from blighted fruit or in the diffusate of such juice for twenty-four to thirty-six hours, some distinct changes could be noted as compared with the appearance of shoots in sap from healthy green pears. The shoots in the latter juice, while somewhat wilted, remained green and showed no discoloration. The drooping and wilting of shoots in juice from blighted fruit has been mentioned. In addition to this, the upper part of the stem and the upper leaves were dark brown to black in color; the lower part of the stem, and the midribs and the veins of the lower leaves, were of a lighter brown color and became blackened after an additional twenty-four hours. This appearance was very similar to that of apple shoots blighted by *B. amylovorus.* It is possible that oxidizing enzymes which are normally present in growing tissue caused the discoloration of the leaves and the stems of apple shoots inserted in the juice from blighted fruit. If this is the explanation, why did not these enzymes manifest themselves in twigs inserted in the juice from healthy green pears?

To ascertain whether other fruit-rotting organisms produced toxic substances similar to those noted in juice from blighted fruit, some green pears were rotted by *Penicillium* sp. and *Sclerotinia cinerea* (Bon.) Wor. and the juice was expressed. No deleterious action was noted when apple shoots were placed in these juices or in their diffusates.

Tests were made to determine whether the substance causing the wilting could be precipitated by alcohol. In these tests, 100 cubic centimeters of juice from blighted pears was diluted with ethyl alcohol until the concentration of alcohol reached 65 per cent. At this point a noticeable amount of brown organic material was precipitated. The solution was filtered through filter paper, and the precipitate was allowed to dry for fourteen hours so that all traces of alcohol were removed. The precipitate was then redissolved in water. Apple twigs placed in the solution wilted in less than two hours.

The filtrate containing 65 per cent of alcohol was further diluted so that the concentration of alcohol became 85 per cent. A very finely divided light brown precipitate became visible. This was allowed to stand for ten hours, the supernatant liquid was then decanted, and the remainder was filtered through a high-grade filter paper. The precipitate, after being allowed to dry on the filter paper for fourteen hours, was redissolved in 50 cubic centimeters of distilled water and the cut ends of young growing apple shoots were inserted in the solution. In two and one-half hours the leaves and the tips drooped, as previously described.

It was thought that perhaps the same toxic action would be exerted by a culture of *B. amylovorus* growing in expressed green-pear juice. The acidity of the sap was adjusted to pH 6.20, 6.68, and 7.80, and some was left at the original concentration of pH 4.07. The cultures thrived in all concentrations except pH 4.07, where growth was uncertain. Green apple shoots placed in these solutions showed no symptoms of toxic action. Similarly, cultures grown in Uschinsky's solution, containing inulin, arbutin, amygdalin, Certo, pectin, and various sugars used for enzyme tests, failed to show any harmful effects on apple shoots. The concentrations of culture media were similar to those used in testing for enzymes, and are given in detail under that topic. Cultures of each of these solutions were tested with apple shoots after forty-eight hours of growth, and again after five days of growth. In some the organisms were killed with germicides or heat, and were tested with apple shoots immediately. Others were allowed to stand for three or four days after being treated, so that the bacteria would lyse and thus release any endotoxins which might be produced. None were found.

The plasmolytic action, or the effect of concentration of the juice from blighted pear and that of the diffusate of such juice, was tested on sections of young green apple stems, leaves, and fruit. The colored epidermal cells of *Tradescantia zebrina* were used also. It was found that little or no plasmolysis occurred except when the juice was highly concentrated. The diffusate caused no marked plasmolysis on any of the tissues tested. This would indicate that the wilting and drooping, with the resultant blackening of stems and leaves, was not the result of too high an osmotic pressure in the pear sap. Even though the wilting and drooping was caused by water loss, the blackening of the stems and leaves could not be explained on this basis.

The property of being precipitated by alcohol would class this substance as being similar in nature to the true bacterial toxins and enzymes. It may be, however, that the toxin is precipitated by being adsorbed by other organic matter, since the amount of organic matter precipitated is rather large at the lower concentration of alcohol, 65 per cent. At the higher concentration of alcohol, 85 per cent, the precipitate is finely divided and is small in quantity, being just sufficient to make the solution turbid.

If the toxic principle contained in juice from blighted pears is in the nature of a true bacterial toxin, one would expect it to dialyze through a thin, but not through a thick, membrane; this was shown to be the case. Also, it should be thermolabile; it appears to be thermostable. Exposure to the air for fourteen hours did not noticeably inactivate the toxic principle; it should have done so. The toxic principle, therefore, may be either an endotoxin or a decomposition product of the host cells. Yet, when lysis of the bacterial cells was produced by heat or by germicides, none of the many artificial cultures in which the organism was grown gave the slightest indication of being toxic for green apple shoots. Also, the sap from green pear fruits rotted by other organisms, failed to cause wilting of apple terminals. Such indirect tests serve to characterize the toxic principle, but its real physical and chemical constitution remains to be determined.

PRODUCTION OF ENZYMES

Microorganisms, both fungi and bacteria, have been shown to be active in the production of enzymes. For a complete review of this subject and the nature of enzyme action, such works as those of Haas and Hill (1921), Waksman (1922), Bayliss (1924), or Waksman and Davison (1926) should be consulted. Waksman and Davison (1926: 108 114) list the enzymes which are produced by bacteria as: catalase, cytase, diastase, emulsin, hemolysins, gelase, invertase, lipase, lactase, maltase, meliases, pyocyanase, oxidases (tyrosinase and luciferase), proteases (gelatinase and casease), reductases, rennet, urease, and probably an enzyme similar to erepsin which causes bacteriophage. Most of the information concerning bacterial enzymes does not relate to phytopathogenic species. However, the enzymes secreted by phytopathogenic fungi have received considerable attention from plant pathologists in studies on parasitism.

In an effort to throw more light on the food requirements and pathogenicity of *Bacillus amylovorus,* a study of the enzymes produced by this organism in artificial media was made.

It is a well-known phenomenon that apple shoots are susceptible to attacks of *B. amylovorus* as long as meristematic tissue is forming, and that as soon as the terminal bud forms the shoots can be repeatedly inoculated with only occasional infection. Experimentally, the host can be placed at the temperature for optimum growth of the parasite, abundant water can be supplied, and still no infections may occur. A possible explanation is that some chemical change has taken place within the host which has made it impossible for the parasite to get food through the action of its enzymes. For example, the phloridzin content of apple twigs as determined by Harvey (1925) shows a close parallel with growth processes. The presence or absence of this compound may be one of the influencing factors in susceptibility or resistance.

Since enzymes cannot be defined in chemical terms and can be detected in a medium only by the products formed, this method of determining their presence was used. If a given product was split by a culture of *B. amylovorus* and such a cleavage did not occur in the uninoculated check, it was assumed that this change was brought about by an enzyme produced by the bacteria. The pH and temperature requirements, the influence of the presence or absence of certain salts, the production of extracellular and intracellular enzymes, and the fact that certain enzymes may be unstable or inactivated in the absence of their substrate or products of their reaction, was carefully considered in each case.

All cultures used for enzyme tests were adjusted to optimum ranges of pH for the respective enzymes, unless otherwise noted. It was noticed that alkaline cultures tended to become more acid, and acid cultures more alkaline. This was especially true of the bouillon and pectic compound cultures.

Carbohydrases

Sucrase, or invertase

Before this work was begun, the only enzyme known to be produced by *Bacillus amylovorus* was invertase, or sucrase, demonstrated by Stewart (1913). He found a moderate amount of this ferment produced in filtered pond water containing 2 per cent of Witte's peptone and 1 per cent of cane sugar.

Uschinsky's solution was made up in accordance with the formula given by Duggar (1909), with the following alteration: 2 per cent of sucrose was substituted for the glycerin, and $CaCl_2$ was omitted. The pH was adjusted to 6.95. In each of six 150-cubic-centimeter Erlenmeyer flasks, 50 cubic centimeters of the solution was placed. Four flasks were inoculated with fire-blight bacteria, the other flasks being reserved as checks. On testing the solutions in the flasks with Fehling's solution for reducing sugars two weeks after inoculation, the following results were obtained:

		Invert sugar (milligrams)	
I.	(a)	156.4	50 cc. 2-per-cent sucrose, with toluol added two days after inoculation
	(b)	159.0	
II.	(a)	229.6	50 cc. 2-per-cent sucrose, inoculated but no toluol added
	(b)	220.3	
III.	(a)	17.4	Checks, not inoculated
	(b)	15.5	

Toluol was added to two of the flasks in order to determine whether any large amount of the enzyme invertase was intracellular. More invert sugar was found where the cultures were permitted to grow than where toluol was added. In two weeks of growth many more bacterial cells would develop and disintegrate than in two days. Probably more cells developed and lysed in two weeks than had been formed in the two-days-old culture at the time when toluol was added, so that it cannot be decided from this test how much invertase, if any, is intracellular. It is evident, however, that under these conditions invert sugar is produced by the bacteria in larger quantities than is necessary for growth.

In each of six tubes, 10 cubic centimeters of Uschinsky's solution, with 2 per cent of sucrose added, was placed. After three of the tubes had been inoculated with *B. amylovorus*, all were incubated as described above for the flasks, and at the end of two weeks the sucrose in all six tubes was inverted and tested with Fehling's solution, following methods outlined by the Association of Official Agricultural Chemists. It was found that the culture tubes contained 28.2 milligrams less invert sugar than did the check tubes. It appears that the bacteria used this sugar in growth processes.

From these tests it is apparent that *B. amylovorus* can split sucrose and utilize the reducing sugars for growth.

Maltase

A solution similar to that used for invertase was employed in testing for maltase, maltose being substituted for sucrose. In each of eight test tubes 10 cubic centimeters of this solution was placed, and this was incubated for ten days at 23° C. after inoculating four of the tubes. At the end of ten days the solution in two uninoculated tubes was tested, without inversion, with Fehling's solution, and it yielded a mean of 198.0 milligrams of maltose; two culture tubes similarly treated yielded 182.4 milligrams of maltose. The maltose in the four remaining tubes was inverted by boiling for two hours with hydrochloric acid. The two check tubes gave a mean of 205.5 milligrams of glucose; the two culture tubes gave a mean of 180.2 milligrams of glucose.

Since glucose has approximately 2.32 times the reducing power of maltose on Fehling's solution, it was thought that perhaps the culture tubes would yield more cuprous oxide than would the check tubes, especially if *Bacillus amylovorus* excretes a considerable amount of maltase. Such was not the case. It is only after inverting the maltose to glucose and determining the total sugar in the solution, that conclusive evidence of maltase formation is found. Apparently the bacteria utilize glucose about as rapidly as it is formed.

The bacteria grew luxuriantly, and this fact would lead to the belief that maltose was utilized. It was found, however, that a small amount of bacterial growth would take place in Uschinsky's solution without sugar, glycerin, or $CaCl_2$. Apparently the bacteria are able to split sodium asparaginate sufficiently to enable them to utilize carbon from that source.

Amylase

Soluble starch was substituted for gylcerin in Uschinsky's solution in testing for amylase. Ten tubes containing 10 cubic centimeters each were prepared, and half of the lot were inoculated. The prepared solution readily reduced Fehling's solution. Aften ten days of incubation the starch was converted to hexoses which reduced Fehling's solution. The results were so variable that no significance could be attached to them. It remains to be demonstrated whether or not *Bacillus amylovorus* can utilize starch through the secretion of amylase.

When Burrill (1882) described the causal organism of fire blight, he named it *Micrococcus amylovorus*, meaning "starch-loving." Most workers since Burrill's time have held that *B. amylovorus* does not digest starch, or if it does the process is very slow. D. H. Jones (1909: 60) found diastasic action on starch jelly after three days at 25° C., but Stewart (1913: 330) was unable to substantiate Jones' claim.

Inulase

In the test for inulase, Uschinsky's solution was prepared using 1 per cent of glycerin. A portion of the solution was inoculated, was incubated for fifteen days, and showed no reducing action on Fehling's solution. To the remainder of the solution 1 per cent of inulin was added, and three flasks containing 40 cubic centimeters each were used for each check and culture. All the flasks were incubated for eleven days at 25° C. Since inulin does not reduce Fehling's solution, levulose present in the culture medium would show the decomposition of inulin. The following results were obtained on testing for sugars:

Culture 40 cc., no toluol, 25.9 milligrams of sugar
Culture 40 cc., toluol added two days after inoculation, 10.5 milligrams of sugar
Check 40 cc., no toluol, 7.2 milligrams of sugar
Check 40 cc., toluol added two days after cultures were inoculated 7.5 milligrams of sugar

Approximately three times as much Cu_2O was precipitated from the Fehling's solution by the culture as by the checks. While the total amount of sugar released from inulin is small, it is nevertheless significant. At the time when the tests were made, the precipitate in the checks was just sufficient to be detected by the eye. The precipitate in the cultures was positive, and appeared to be greater than subsequent weighings showed. The writer is convinced that *Bacillus amylovorus* can split inulin, and, if this is accepted, then it may be inferred that inulase is secreted, unless the splitting of inulin is attributed to the presence of sucrase.

Pectinase

The enzyme pectinase is often considered as a group of three distinct enzymes: pectosinase, pectase, and pectinase. According to Atkins (1916), pectosinase is the enzyme which converts the pectic compounds of the middle lamella into soluble pectin; pectase coagulates pectin; and pectinase hydrolyzes pectin to reducing sugars of the pentose series. Not all authors agree on the preceding classification; some believe there is but one enzyme acting on pectic compounds. However, for the sake of clarity, the writer will use Atkins' terminology.

Stewart (1913: 360) failed to find evidence of any cell wall dissolving enzymes secreted by *Bacillus amylovorus*. Nixon (1927) found that when *B. amylovorus* was migrating in the host tissue as zoogloea, there was little or no dissolution of lamellae or cell walls; but when the bacilli changed phases and began to form lysigenous cavities, both cell walls and lamellae were broken down.

The writer placed thin slices of unripe pear and apple fruits in bouillon cultures of the fire-blight organism. Similar slices were placed also in boiled and in fresh juices extracted from blighted pear fruits as described in the discussion of toxins (page 14). In addition, pieces of these infected fruits used for the toxin experiments were fixed in chrom-acetic acid and sectioned in paraffin. In none of these tests did a dissolution of the middle lamella occur prior to disintegration of the entire tissue. In working with this material, the walls of the cells were broken more frequently than they were split. This would indicate that if any dissolution of the middle lamella occurs, it does so very slowly. Also, the fact that blighted fruits and twigs are firm and are not easily reduced to pulp by squeezing, lends support to this view. From this it may be concluded that a lamella-dissolving enzyme or pectosinase is secreted only in minute quantities, if at all, by *B. amylovorus* under the conditions of the experiments.

In testing for pectase and pectinase, pectin was prepared from Certo, a commercial apple pectin, by coagulating with ethyl alcohol, filtering, washing with cold water, redissolving in warm water, and recoagulating. This process was repeated three times, and the pectin thus obtained was dissolved in warm Uschinsky's solution from which the glycerin had been omitted. The pectin was used at the rate of about 10 per cent by volume. Tests for reducing sugars in this preparation proved to be negative. All tubes thus prepared were incubated at 25° C. for two days and tested for sterility. Nineteen tubes were divided into two lots, lot 1 being inoculated with a culture of *B. amylovorus* and lot 2 serving as a check. The tubes were examined daily for signs of coagulation. No such signs were noted during a period of twelve days. At the end of that time all tubes were tested for reducing sugars. If the enzyme pectinase was formed, some of the soluble pectin should have been hydrolyzed to the reducing sugar arabinose. No reducing sugars were detected. If arabinose had been detected and no coagulation noted, the test for pectase would not have been positive, as the enzyme pectinase is able to hydrolyze soluble pectin and also the gel formed by pectase. Conversely, pectase is without effect on pectic bodies previously hydrolyzed by pectinase, according to Bourquelot and Hérissey (1895).

Since arabinose was not found in the cultures, nor any evidence of coagulation detected, it must be inferred that under the conditions of the tests neither pectase nor pectinase was produced.

Cellulase

Tests for the production of cellulase were made by placing pieces of Reeve's Angel filter paper of known weight in Uschinsky's solution containing 0.5 per cent of glycerin, and in similar solutions without glycerin. Ten culture tubes and five checks were used for each concentration. After fifteen days, tests were made for sugars but none were found. There was no significant loss in the weight of the paper.

Half of the cultures were continued for another ten days after 2 per cent of toluol had been added. Negative results were again obtained. Two additional tests duplicating the conditions of the first failed to give any indication of the presence of cellulase.

From these results it must be concluded that cellulase was not secreted under the conditions of the experiment. Histological studies of diseased tissue support the view that *Baccillus amylovorus* produces little or no cellulase.

Tests for "cytase," which according to Haas and Hill (1921) is the enzyme that splits hemicelluloses, were not made. Since Schellenberg (1908) and Zeller (1916) found this enzyme present in phytopathogenic fungi, it would not be surprising to find it in pythopathogenic bacteria.

Glucosidases

Amygdalase

Emulsin was formerly supposed to split glucosides such as amygdalin into glucose, benzaldehyde, and hydrocyanic acid. According to Haas and Hill (1921), there are two enzymes which take part in this process: amygdalase in the first step, and prunase in the second. Maltase also is credited with the power to split amygdalin into glucose and mandelonitrile glucoside, the same as amygdalase.

Waksman and Davison (1926) used the term *emulsins* to include all enzymes which are capable of hydrolyzing glucosides and various sugars such as raffinose and stachiose. Emulsin is an enzyme preparation rather than a single enzyme. Oppenheimer (1925) has attempted to divide the emulsins on the basis of the glucosides upon which they act.

Tests were made with *Bacillus amylovorus* to determine whether amygdalase or prunase is secreted by this organism. Uschinsky's solution was prepared with 1 and 2 per cent of amygdalin replacing the glycerin. Six check tubes and six cultures of each concentration were prepared and incubated at 25° C. for fourteen days. It was apparent throughout this time that the cultures in the 1-per-cent amygdalin made better growth than did those in the 2-per-cent solution.

At the end of fourteen days, 2 per cent of toluol was added to half of the tubes, which were stored at about 3° C. The remainder were tested for the reducing sugar glucose, but none was found. There was no odor of almond to betray the presence of benzaldehyde. Tests for hydrocyanic acid failed to give any indication of the formation of prussian blue.

Seven days after these tests were made, the tubes containing the toluol were examined. No traces of glucose, benzaldehyde, or hydrocyanic acid were found. The tests were performed in triplicate with similar results. From this it must be concluded that amygdalase was not produced.

The presence of maltase was evident when maltose was used, but this enzyme failed to appear, or at least to exert any influence in the media, when amygdalin was substituted for maltose. Waksman (in Jordan and Falk, 1928:270) makes the following statement: "The presence of specific nutrients in the medium regulates the quantitative formation of specific enzymes. Thus, the presence of starch will increase the amylolytic power; the presence of proteins, the proteolytic power, etc." The pH of both cultures was adjusted to 6.18, which is about the optimum for both enzymes. Either maltase was not formed, as Knudson (1913) found with tannase, or the enzyme is unstable in the absence of its substrate or products of its reaction, a hypothesis advanced by Professor O. F. Curtis, of Cornell University, to explain the increased amount of a given enzyme found in a culture supplied with the specific substrate. Maltase is known to be unstable above 0° C.

Arbutase

Cultures similar to those used with amygdalin were made with arbutin. After sterilization the medium was clear, resembling distilled water in appearance, and gave negative results for sugar tests.

After five days the culture tubes became turbid and had a faint brown color. At the end of fifteen days the brown color was very distinct, indicating the formation of hydroquinone. Tests for sugar again proved negative. To half of the cultures and checks, 2 per cent of toluol was added. Six days later the cultures were distinctly darker, and tests for reducing sugars proved positive. The same results were obtained in two subsequent tests. The checks remained clear throughout and gave no evidence of the formation of hydroquinone or of reducing sugars.

What enzyme caused this reduction in the culture tubes? If amygdalase was produced there should have been some reduction in the cultures containing amygdalin. No evidence is found in the literature that arbutin is hydrolyzed by maltase, as is stated for amygdalin. We must accept either the facultative or qualitative production of enzymes, or the view that enzymes which are specific for glucosides are produced (Oppenheimer, 1925) as are enzymes which are specific for the various sugars. If the latter view is adopted, then we shall have to say that *Bacillus amylovorus* produces the enzyme arbutase, in conformity with the terminology used for enzymes.

Arbutin is the predominant glucoside in pear shoots according to Lincoln (1926). Phloridzin, the principal glucoside in apple, is found also in plum, sweet cherry, and probably Spiräea, Crataegus, and Amelanchier according to Harvey (1925). Harvey shows a rather close correlation between rapidity of growth and arbutin and phloridzin content in the pear and apple shoots. This may have a direct bearing on susceptibility to infection by *B. amylovorus.* Whether or not the fire-blight organism can hydrolyze phloridzin remains to be demonstrated, but the evidence is at least suggestive.

Protease

Trypsin

Blood fibrin was stained with 1 per cent of congo red, boiled in water to fix the color, and then autoclaved with Uschinsky's solution without glycerin. Some color was released by the autoclaving. The colored solution was decanted, sterile solution was added, and, after incubating for forty-eight hours to test for sterility, eight of the sixteen tubes were inoculated and the remainder were reserved as checks. Growth of the cultures for three weeks failed to show any digestion of the fibrin with a resultant release of red color. This would indicate that *Bacillus amylovorus* did not produce trypsin in these tests.

Summary on enzymes

Summing up the writer's work on the enzymes produced by *Bacillus amylovorus,* the following were found: sucrase, or invertase, maltase, inulase, and arbutase. Tests for amylase, pectosinase, pectase, pectinase, cellulase, amygdalase, and trypsin failed to give positive results. These tests do not, of course, demonstrate the inability of *B. amylovorus* to produce any or all of the latter group of enzymes. Different methods may show later that the enzymes were merely inhibited in formation or inactivated in these experiments.

LONGEVITY OF THE ORGANISM

Difference of opinion exists among pathologists as to the ability of *Bacillus amylovorus* to withstand adverse conditions. Both D. H. Jones (1909) and Stewart (1913) found that drying for five days on a cover glass did not kill the organism. However, Stewart obtained no growth after seven days in bouillon from organisms so treated. In attempting to solve the problem of rapid dissemination of *B. amylovorus* in an orchard, Gossard and Walton (1922), of Ohio, showed that the organism can live in honey for seventy-two hours or more, in aphid honeydew for seven to ten days if moisture is supplied, and in nectar of peach, plum, and cherry, for five days or longer. The last-mentioned fact is not surprising, as both plum and cherry have been proved to be hosts for the organism. All attempts to isolate *B. amylovorus* from the soil have met with disappointment.

It was early recognized that after twigs or branches were cut from a tree, the bacteria died, in a majority of the branches, in a few days. Arthur (1887 b) was one of the first to make tests on the longevity of the fire-blight organism. He found viable bacteria in some infected branches nine months after they had been removed from the tree and thrown into an open cistern. No inoculations are recorded in which the bacteria isolated from the inner bark of these branches were used. Fulton (1911), in Pennsylvania, reported that of thirty-five pruned twigs which were left lying on the ground, thirty-one contained no living bacteria after seven days. Hotson (1916), in Washington, extended the time that fire-blight bacteria may live in pruned branches to a month. A limb placed in the sun in his laboratory, where the temperature during the day was usually from 40° to 50° C., contained viable fire-blight organisms for twenty-eight days. Waters (1922) has shown that living bacteria were recovered from dried exudate after a period of nine months. In Colorado, Sackett (1911) found approximately 25 per cent of the eighty-three cankers which he examined to be "hold-over" cankers. Stewart (1913: 346) reports isolating the organism from pear twigs less than 1 centimeter in diameter, and from pear-seedling trunks about ½ inch in diameter, during the dormant season. Dr. H. E. Thomas examined more than one hundred small twig cankers of apple in 1923 about blossoming time, and found no living fire-blight bacteria.

Observations on "hold-over" blight were made by the writer in 1925 and 1926 at blossoming time in an apple orchard near Ithaca. The results of these observations are presented in table 4. Cultures were

TABLE 4. "HOLD-OVER" CANKERS OF BACILLUS AMYLOVORUS ON APPLE AT BLOSSOMING TIME

Date	Variety of apple	Cankers viable or dead	Diameter of branches				
			¼ inch	⅜ inch	½ inch	⅝ inch	¾ inch
May, 1925	King	Number dead...	56	40	28	20	14
		Number viable..	3	5	6	9	4
		Per cent viable..	5.1	11.1	17.6	31.0	22.2
May, 1925	McIntosh	Number dead...	72	54	21	5	2
		Number viable..	0	1	1	0	0
		Per cent viable..	0	1.8	4.5	0	0
June, 1926	King	Number dead...	42	37	18	11	6
		Number viable..	2	3	2	2	3
		Per cent viable..	4.5	7.5	10.0	15.4	33.3
June, 1926	McIntosh	Number dead...	64	58	18	7	3
		Number viable..	0	2	1	1	0
		Per cent viable..	0	3.3	5.3	12.5	0

made from six of the twigs measuring ¼ inch in diameter, to verify the conclusions reached by the observations. In each case the isolations checked with the observations.

From the foregoing data it is evident that small twigs may serve as "hold-overs" for fire blight. In Plate II, 3, drops of ooze which are sometimes found on these small twigs are shown. It is at once apparent that even though only a small number of twigs might serve as "hold-overs," this fact would seriously handicap any eradication campaign.

Except when directly associated with a living part of the host plant, the bacteria in nature are generally believed to die rather quickly. The reports of Arthur, Hotson, and Waters, already noted, do not substantiate this belief. Dr. Thomas obtained virulent organisms twelve months after infected limbs had been brought into his laboratory. Dr. W. H. Burkholder, of the Cornell station, working with *Bacterium flaccumfaciens* Hedges, obtained pathogenic bacteria from herbarium specimens (leaves of *Phaseolus angularis*) five years old.

A number of yearling Transcendent crab-apple trees which were infected with *Bacillus amylovorus* and showed large drops of ooze, were brought into the laboratory by the writer in May, 1925. Cultures made from the dried ooze at three-months intervals yielded the organism each time for two years and three months. The last cultures, at two and one-half years, failed to produce the organism, but it is possible that the failure was due to technic rather than to loss of viability of the organism. The initial bouillon cultures were allowed to incubate for four days before dilutions were made, and were heavily charged with contaminating organisms.

Neither *B. amylovorus* nor any other bacterial plant pathogene is known to form endospores. Yet it seems almost unbelievable that vegetative forms of bacterial cells will live for five years on dried herbarium specimens or for two years as dried ooze on apple twigs. Of course these conditions do not duplicate those of the field, especially in regard to rapid fluctuations in temperature and humidity, and to sunshine.

The work of Nixon (1927) suggests that there may be resting forms of these organisms, as cysts or involute forms, which may be more resistent to adverse conditions and may approximate in function and characteristics the endospores formed by such species as *Bacillus botulinus*. The nature and properties of the matrix in which the bacterial cells are embedded in the ooze deserve further study in this connection.

MODE OF ENTRANCE INTO BLOSSOMS

Arthur (1886: 272), whose three years of work on fire blight (1884 to 1886) were begun shortly after Burrill discovered the causal organism, concluded that "the germs of the disease gained entrance to the interior of the branches through the newest growth of the young shoots and the central parts of the flower, especially the moist nectariferous surfaces." Waite (1896:298) suggests that bees, wasps, and flies undoubtedly carry the microbe from oozing cankers to the blossoms, and states (page 297 of reference cited) that the microbes "are able to live and multiply in the nectar of the blossom." The last-mentioned fact has been observed by other workers and unquestionably substantiated by Gossard and Walton (1922).

In order to determine whether infection would occur when other parts of the blossom than the nectaries were inoculated with the fire-blight organism, a number of branches of Rhode Island Greening apples and Bartlett pears were brought into the greenhouse in the latter part of March, 1925, and placed in battery jars containing water. In about two weeks the branches blossomed profusely, and on April 14 inoculations were made with a bouillon culture of *Bacillus amylovorus* in the following ways: (1) by brushing the center of each petal with a camel's-hair brush; (2) by puncturing the center of each petal with a needle; (3) by puncturing the base of the blossom with a needle; (4) by brushing the stigmas with a very fine camel's-hair brush; (5) by atomizing into the center of the blossom. The results of the inoculations are given in table 5.

The average temperature of the greenhouse was about 65° F. No insects were noticed on the branches. Aphids were abundant on the opening buds but were killed by fumigation shortly before the blossoms opened. Results similar to those shown in table 5 were obtained by another series of inoculations made in the latter part of April.

It was recognized that the conditions of the cut branches, and their environmental influences, were entirely unlike the conditions prevailing in nature. For this reason, similar tests were made during the blossoming period in the Preswick and the Slater Brothers' orchard, at Ithaca and Rochester, New York, respectively. In addition to bouillon cultures, fresh bacterial ooze also was employed. Inoculations were made on branches covered with cheesecloth bags. Some inoculations were made also on unprotected blossoms, and comparable results were obtained but they are not recorded here since there is no assurance that insects did not inoculate these blossoms. The data are recorded in table 5. The weather records covering the blossoming period for the Preswick and the Slater Brothers' orchard in 1925 are given in table 9 (page 42).

Again, in 1926, stigma and petal inoculations were made in the Preswick orchard and the Slater Brothers' orchard (table 5). The blossoms were inclosed in glassine bags, to prevent the possibility of rain wash-

TABLE 5. RESULTS OF BLOSSOM INOCULATIONS

Date of inoculation	Location	Variety of fruit	Method of inoculation	Number of blighted blossoms	Number of healthy blossoms	Per cent blighted
April 14, 1925	Cornell University greenhouse	Bartlett pear	Petal brushed	32	18	64.0
		Rhode Island Greening apple	Petal brushed	35	15	70.0
		Rhode Island Greening apple	Petal punctured	27	8	77.1
		Bartlett pear	Petal punctured	22	13	62.8
		Bartlett pear	Receptacle punctured	50	0	100.0
		Rhode Island Greening apple	Receptacle punctured	48	2	96.0
		Rhode Island Greening apple	Stigma brushed	23	12	65.7
		Bartlett pear	Stigma brushed	19	16	54.3
		Bartlett pear	Atomized	75	0	100.0
		Rhode Island Greening apple	Atomized	74	1	98.7
		Rhode Island Greening apple	Check (not inoculated)	0	68	0.0
		Bartlett pear	Check (not inoculated)	0	102	0.0
May 18, 1925	Preswick orchard	Tompkins King apple	Petal brushed; bouillon	0	25	0.0
		Tompkins King apple	Petal punctured; bouillon	0	26	0.0
		Tompkins King apple	Petal brushed; bouillon	0	25	0.0
		Tompkins King apple	Petal punctured; bouillon	0	25	0.0
May 25, 1925	Slater Brothers' orchard	Rhode Island Greening apple	Petal brushed, ooze	0	26	0.0
		Rhode Island Greening apple	Petal punctured; ooze	25	13	65.8
May 26, 1925	Slater Brothers' orchard	Rhode Island Greening apple	Petal brushed; ooze	9	36	20.0
		Rhode Island Greening apple	Petal brushed; bouillon	0	27	0.0
		Rhode Island Greening apple	Petal punctured; ooze	29	15	65.9
		Rhode Island Greening apple	Petal punctured; bouillon	0	28	0.0
		Rhode Island Greening apple	Stigma brushed	29	10	74.3
		Rhode Island Greening apple	Stigma brushed; ooze	15	9	62.5
		Rhode Island Greening apple	Check (not inoculated)	0	55	0.0
May 30, 1926	Preswick orchard	Tompkins King apple	Petal brushed; bouillon	4	70	5.4
		Tompkins King apple	Stigma brushed; bouillon	6	81	6.9
		Tompkins King apple	Atomized; bouillon	47	3	94.0
		Tompkins King apple	Check (not inoculated)	0	50	0.0
June 4, 1926	Slater Brothers' orchard	Tompkins King apple	Stigma brushed; bouillon	6	97	5.8
		Tompkins King apple	Petal punctured; bouillon	0	45	0.0
		Tompkins King apple	Petal brushed; bouillon	0	70	0.0
		Tompkins King apple	Atomized; bouillon	42	8	84.0
		Tompkins King apple	Check (not inoculated)	0	60	0.0
		Rhode Island Greening apple	Stigma brushed; bouillon	8	116	6.4
		Rhode Island Greening apple	Petal punctured; bouillon	0	90	0.0
		Rhode Island Greening apple	Petal brushed; bouillon	0	50	0.0
		Rhode Island Greening apple	Atomized; bouillon	46	4	92.0
		Rhode Island Greening apple	Check (not inoculated)	0	51	0.0

ing the inoculum from the petals and stigmas into the nectaries and to prevent insect visitation.

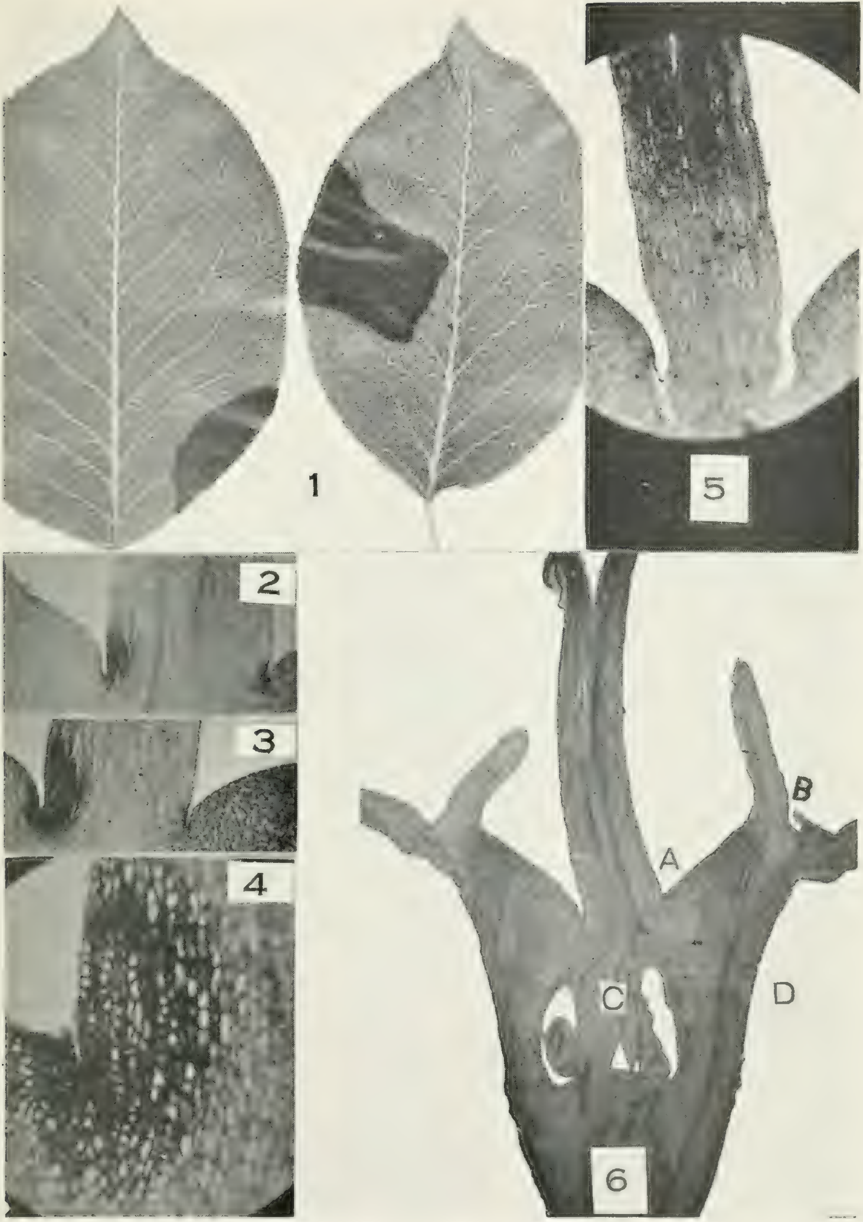
In some blossoms, only two or three styles were infected by the blight organism. About two days after inoculation the first symptoms of disease were noted. The infected stigmas and styles were dark green and appeared to be water-soaked. Only the upper third or half of the style showed this symptom. In about four days after inoculation, the stigma and the tip of the style were dark brown and began to wither, while the part nearest the ovary was dark green. Sections through such blossoms, and cultures from the various parts, showed the ovaries to be healthy and the style to be invaded by bacteria. There was a progressive killing of the tissues from the stigma to the ovary. Styles not infected remained healthy until the ovaries were invaded. Infected petals formed a V-shaped light brown area, with the apex at the point of inoculation and the base at the margin of the petal. In many cases the petal became infected but fell before the bacteria could invade the receptacle.

The results indicate that, under greenhouse conditions, infection of pear and apple blossoms by the fire-blight organism can take place through the petals without wounding the surface, and that infection can result from inoculation of the stigmas. The same conclusion can be drawn for the outdoor conditions, but the percentage of infection resulting from inoculating either the petals or the stigmas is markedly decreased as compared with cut twigs in the greenhouse.

While the percentage of blighted blossoms was small in some of the experiments, there is no question that infection can take place through both the petals and the stigmas. Isolations were made from the ovaries of the blighted blossoms and a virulent culture was obtained. However, it is not likely that a high percentage of blossoms are inoculated through either the stigmas or the petals under normal conditions.

In order to determine what tissues are invaded most frequently by the fire-blight bacteria, and to gain further knowledge of blossom infection, apple blossoms were inoculated by atomizing with bouillon culture, and at intervals of twenty-four hours the blossoms were fixed in chrom-acetic acid fixer, embedded in paraffin, sectioned, and stained with Fleming's triple stain. The blossoms were removed from the trees just preceding the fixing. Cheesecloth bags inclosed the blossoms from the time of the early pink stage.

Progressive killing of the tissue twenty-four, seventy-two, and one hundred and forty-four hours after inoculation is shown in Plate III, 2, 3, and 4. The point most frequently attacked appears to be the nectariferous region (Plate III, 6, A). Gossard and Walton (1922) have shown that the organism can live in nectar. With the nectar as a source of food supply, the bacteria would be able to multiply rapidly and enter the interior of the blossom by means of either enzymic or toxic secretions or both. That the bacteria should attack the base of the style and progress more rapidly toward the center of the receptacle (Plate III, 6, from A toward C) than outward toward the base of the stamens and the petals (Plate III, 6, from A toward B), is logical, since the fire-blight organism thrives best in rapidly growing tissue. No cases were found in which the opposite was true. Killing the tissue of the receptacle from the nectar glands toward the ovaries would soon kill the entire flower. This appears to be the



LEAF AND BLOSSOM INVASION BY BACILLUS AMYLOVORUS

1, Leaf invasion on pear foliage
2–4, Successive stages in blossom invasion at 24, 72, and 144 hours after inoculation (× 50, 50, and 215, respectively)
5, Blossom invasion through the style (× 50)
6, General view of apple blossom in section (× 12)

mode of functioning of the bacteria in most instances under natural conditions. That occasionally the bacteria may gain entrance through the stigmas is indicated. Killing of the style by the bacteria while the remainder of the flower is healthy is shown in Plate III, 5. No infection is apparent in the nectariferous region. In approximately forty-eight hours more, the bacteria would have reached the base of the style, judging from previous invasion. This substantiates some of the results obtained by stigma inoculations, given in table 5. In none of the sections made by the writer did infection and invasion occur through the epidermis of the receptacle below the sepals (Plate III, 6, region D). This, however, does not preclude such a possibility, especially when the opening flowers are infested with aphids. Needle punctures with bouillon produce disease readily in this region of the flower. Miller (1929) records infection through the sepals of unopened apple flowers.

LEAF INVASION

In 1915 Heald reported that fire-blight bacteria had been found invading apple leaves. He expressed the opinion "that the bacteria enter the intercellular spaces through the water-pores and also by the stomata to some extent . . ." Later (1927) he presented additional notes on natural leaf infection, and reported experiments in which inoculations on pear and apple caused both marginal and central leaf lesions similar to those observed in nature. Hotson (1916) found many pear leaves infected on the Pacific coast, but observed few twig infections resulting from leaf infection. Stewart (1913: 339) figures two pear shoots on which some of the leaves have blighted, owing, he says, to infection at the base of the petiole. Possibly the bacteria gained entrance through the leaves instead of in the manner described by Stewart.

Brooks (1926: 694), working in Wisconsin, reached the following conclusion: "The nectaries of blossoms, and possibly the hydathodes of very young leaves, seem to be the only natural openings through which blight infection of apple takes place. All attempts to secure stomatal infection failed." He found that infection would take place through wounds in leaves up to thirty-six hours after injury. Miller (1928), working in the same laboratory, states that he "has induced at will abundant infection of young shoots and unopened blossom buds of apple and pear by spraying them with a suspension of the fire-blight bacteria . . ." He finds that the organism will also enter the stomata of young leaves and cause infection. Tullis (1929) reports successful twig inoculation on apple and pear by first sprinkling the seedlings for five hours, then placing a drop of inoculum on the leaves and sprinkling overnight.

Leaf infection of pear by *Bacillus amylovorus* was very common in a nursery near Rochester, New York, in 1925, and was found also to a lesser extent at Ithaca. Stewart and Leonard (1916) have shown that *Empoasca mali* Le Baron may act as an inoculating agent of *B. amylovorus.* This species of leaf hopper was unusually abundant on pear and apple trees in the nursery in 1925. Field observations in New York failed to show that leaf infection resulted in twig infection in many cases. Usually the organism died before reaching the base of the petiole. This is in agreement with the observations made by Hotson (1916). Some leaf infection on Bartlett pears is shown in Plate III, 1.

In order to test further the hypothesis of Heald, a number of apple seedlings about 1 foot in height, growing in the greenhouse, were atomized in lots of thirty. Immediately after atomizing, twenty-five of the plants were placed in a humid chamber in the headhouse where the temperature was about 20° C. Five plants were allowed to remain on the greenhouse bench (at about 21° C.) together with three plants that had been punctured with a sterilized needle dipped in the bouillon culture to serve as a check on the pathogenicity of the culture.

This test was repeated six times, a total of one hundred and eighty plants being atomized. The check plants became diseased in all series. All the other plants remained healthy, with the exception of three in the first series atomized on February 23, 1925. At this time the writer noted an abundance of whiteflies (*Trialeurodes vaporariorum* Westw.) infesting the seedlings, but no other insects were found. In the other series, precaution was taken to start the experiment shortly after the greenhouse had been fumigated so that insects would not serve as inoculating agents.

In June, 1926, one hundred and ten pear seedlings, originating from Kieffer seed growing in a nursery row at Ithaca, were atomized one evening about sundown. The plants varied from extremely susceptible to fairly resistant individuals, were from 1 to 3 feet in height, and averaged more than three hundred leaves per plant (Thomas, 1927–28). No leaf infection was noticed on any of the plants, although later in the season some of the seedlings were entirely killed by the fire-blight organism inoculated into the terminals.

In both the foregoing experiments an attempt was made to provide optimum conditions for stomatal or hydathodal infection, but the results were negative. This does not preclude the possibility of such an occurrence, but indicates that leaf infection, like twig infection, is most common through some wound such as an insect puncture. It is doubtful that leaf infection through natural openings plays a very important part in dissemination of fire blight under New York conditions.

RATE OF MIGRATION IN THE SUSCEPT

Many fruit growers and pathologists who have attempted to combat fire blight in orchards are of the opinion that the disease progresses at a rapid rate. This is probably owing to the sudden appearance of symptoms on blasted blossoms or blighted twigs. The method of migration has been studied by Bachmann (1913) and by Nixon (1927), both of whom hold that it is largely intercellular; some difference of opinion exists, but in the main their findings are similar. The writer's tests were made on the *rate* of migration. Waite (1896) states: "It rarely extends farther than 2 or 3 inches from the point of attack in one day, but occasionally reaches as much as 1 foot." The basis for this statement is probably observation, as no supporting data are given. Whetzel and Stewart (1909) thought that the bacteria could not penetrate more than a few inches a day, even under the most favorable conditions. D. H. Jones (1909) stated that the disease "may travel down the twig at the rate of from a quarter of an inch to two inches a day, the rate of its progress depending largely upon the succulency of the twig and the atmospheric temperature." That both temperature and succulency are factors in rate of migration was proved by Brooks (1926), who added humidity also as a limiting factor. He found that the rate of migration at optimum temperature of 21° to 28° C. varied from 0.33 to 1.25 inches a day; at optimum relative humidity (80 per cent), the rate varied from 0.19 to 1 inch a day. The rate in twigs of ordinary growth at 50 per cent relative humidity was as low as 0.07 inch a day; and at 10° C. it was as low as 0.07 inch a day. Brooks, however, did not report using optimum temperature and optimum humidity in any controlled experiments.

The writer used apple trees growing under greenhouse conditions with a temperature range from about 65° to 95° F. The method employed was briefly as follows: French seedling apple trees were grown in the greenhouse and were tested for their susceptibility to the fire-blight organism. After a rest period out of doors, the plants were dug up, potted, and given a heavy application of sodium nitrate to induce rapid growth. The plants selected for inoculation had formed new growth from 20 to 25 inches in length. Inoculations were made, about 1 inch from the tips of the shoots, by puncturing with a hypodermic needle and injecting a drop of bouillon culture seventy-two hours old. The same culture was used for all plants in a series. Twenty-four hours after inoculation, the tips of six plants were cut off at 1, 2, and 7 inches, respectively, below the point of inoculation, two plants being used for each distance. Inoculation and removal of the tips was performed at nine o'clock each morning. On the second day—forty-eight hours after inoculation—eight tips were removed, at

TABLE 6. RATE OF MIGRATION OF BACILLUS AMYLOVORUS*

Date of inoculation (9 a. m.)	Date of cutting (9 a. m.)	Distance cut below point of inoculation, in inches												
		1	2	3	4	5	6	7	8	9	10	12	14	16
May 11, 1925	May 12	– –	– –	....	....	....	....	– –	....	....	....	....	....	....
May 11, 1925	May 13	+ –	– –	....	– –	....	....	– –	....	....	....	....	....	....
May 11, 1925	May 14	....	+ +	+ –	– –	....	....	– –	....	....	– –	....	....	....
May 11, 1925	May 15	....	+ +	....	+ –	....	....	– –	....	....	– –	– –	....	....
May 11, 1925	May 16	....	....	+ +	....	+ –	....	– –	....	....	– –	– –	....	....
May 11, 1925	May 17	....	....	+ +	+ –	....	....	– –	....	....	– –	– –	....	....
May 11, 1925	May 18	....	....	+ +	....	+ +	....	+ –	....	....	– –	– –	....	....
May 11, 1925	May 19	....	....	+ +	....	+ –	....	+ +	....	+ –	+ –	– –	– –	– –
February 22, 1926	February 23	– –	– –	– –	....	....	....	....	....	....	....	....	....	....
February 22, 1926	February 24	+ –	– –	– –	– –	....	....	....	....	....	....	....	....	....
February 22, 1926	February 25	+ +	+ –	....	+ –	....	– –	....	....	....	....	....	....	....
February 22, 1926	February 26	....	+ +	....	+ +	....	– –	....	– –	....	....	....	....	....
February 22, 1926	February 27	....	+ –	....	+ +	....	– –	....	– –	....	– –	....	....	....
February 22, 1926	February 28	....	....	....	+ +	....	+ –	....	+ –	....	– –	–	....	....
February 22, 1926	March 1	....	....	....	+ +	....	+ +	....	+ –	....	+ –	– –	– –	– –
June 5, 1926	June 6	– –	– –	....	....	....	....	....	....	....	....	....	....	....
June 5, 1926	June 7	– –	– –	– –	....	....	....	....	....	....	....	....	....	....
June 5, 1926	June 8	....	+ +	+ –	+ –	....	....	....	....	....	....	....	....	....
June 5, 1926	June 9	....	+ +	+ +	+ +	....	– –	–	....	....	....	....	....	....
June 5, 1926	June 10	....	....	+ +	+ +	....	+ –	....	+ +	....	– –	–	....	....
June 5, 1926	June 11	....	....	+ +	+ +	....	+ –	....	+ –	....	– –	– –	–	....
June 5, 1926	June 12	....	....	....	+ +	....	+ +	....	+ +	....	+ –	– –	– –	– –

*+ indicates infection below point of cutting. – indicates no blighting below point of cutting. Each + or – symbol represents a single tree.

1, 2, 3, and 4 inches, respectively, two plants again being used for each distance. This procedure was continued for seven or eight days, at the distances indicated in table 6. Eight days later the plants were inspected, to determine from which ones the blight had been removed and those in which infection had progressed beyond the point of cutting. If killing had progressed after cutting, it was assumed that the fire-blight pathogene had passed that point before the cut was made. If no further killing had occurred, it was assumed that all bacteria were removed when the twig was severed. That fire-blight bacteria will not migrate at the same rate in all shoots is to be expected, but some correlation in the rate at which invasion progresses is shown in table 6.

It appears that immediately after inoculation, some time is required for the marshaling of the bacterial forces before their invasion of new tissue. After migration has begun, the rate is fairly uniform for the first seven days, for the three series. Variations exist in the individual series, which may be due to changes in atmospheric temperature or in relative humidity, or to variation in the growth processes of each host plant.

EPIPHYTOLOGY

DISSEMINATION OF THE ORGANISM

Both Burrill (1881 b) and Arthur (1885 a, 1886) speculated on the mode of spread of the fire-blight bacteria. Arthur (1885 a) expressed the opinion that the bacteria, after being washed about, dried up, were then blown about by the wind, and, alighting on the "delicate surface tissues of expanding buds, or the exposed internal tissues of fresh cracks or wounds," caused the disease to develop. He noted a progression of blight from the direction of the prevailing wind. He was "not inclined to consider" insect transmission as a "very frequent means of contagion." Waite contributed the first experimental evidence on insect transmission in 1898, although three years earlier he had stated that bees, wasps, and flies are attracted to the exuding bacterial ooze and undoubtedly carry the microbe to the opening blossom.

The rôle of bees in dissemination was more recently investigated by Gossard and Walton (1922), who found that *Bacillus amylovorus* can live in honey for seventy-two hours or more, and who were able to produce killing of apple shoots, attributed to fire blight, by inserting the mouth parts of bees into such shoots during blossoming time. That many species of insects may transmit fire blight can hardly be questioned when one reads, in addition to the references mentioned, the reports of D. H. Jones (1909), Whetzel and Stewart (1909), O'Gara (1908, 1910), Stewart (1913), Stewart and Leonard (1916), Smith (1920), and Brooks (1926). That the blight organism may be transmitted by the pruning knife or by other tools has been proved by Whetzel (1906), Stewart (1913), and others. Pickett (1914), Gossard and Walton (1922), Talbert (1925), Brooks (1926), Miller (1928), Tullis (1929), and Rosen (1929) think that meteoric water plays an important part in dissemination, by washing the bacteria either into wounds or over blossoms and young, tender, growing shoots. Stevens and others (1918) suggest support of Arthur's hypothesis of wind dissemination. Thus we find a diversity of opinion as to which agent or agencies bring about the rapid dissemination of fire-blight bacteria. That in different regions and in different seasons the percentage of spread attributable to any one agency may vary greatly, is indicated.

Following the announcement by Gossard and Walton (1922) in Ohio that rain acts as a vector of *B. amylovorus*, tests were made in New York to determine whether this method of dissemination might be one of the contributing factors in causing epiphytotics of fire blight under conditions prevailing in this State. In order to exclude insects and not rain, ten coarse cheesecloth bags (22 meshes to the inch), each one-half yard wide and approximately one yard long, were tied on branches of Tompkins King apple trees at Ithaca on May 14, 1925. The blossom clusters had just begun to unfold. After a few days, when some of the blossoms in the bags and on other parts of the tree had opened, approximately one hundred blossoms immediately above each bag were atomized with a virulent culture of *B. amylovorus*. The blossoms in the bags were protected from accidental inoculation by the bouillon during atomizing, since preliminary tests had shown that these blossoms could be inoculated with an atomizer through the cloth. In the course of a week to ten days there was a semicircle of blighted blossoms above each bag. The number of blighted blossoms in the bags is shown in table 7. The precipitation and temperature records during these tests are given in tables 9 and 11 pages 42 and 44).

TABLE 7. INFECTION IN BAGGED BLOSSOMS BY BACILLUS AMYLOVORUS

Date of starting experiment	Location	Variety	Number of bags	Number of blossoms	Number of blossoms blighted
May 14, 1925	Ithaca	Tompkins King	10	390	0
May 21, 1925	Rochester	Tompkins King	4	190	5*
May 21, 1925	Rochester	Rhode Island Greening	4	186	0
May 21, 1926	Ithaca	Wagener	13	936	0
May 29, 1926	Ithaca	Tompkins King	14	735	0
June 4, 1926	Rochester	Tompkins King	10	655	0
June 4, 1926	Rochester	Rhode Island Greening	11	845	0
Total			66	3,937	5

* These five blossoms were all in one cluster and were appressed to the cheesecloth, and could easily have been inoculated by an insect through the meshes of the cheesecloth.

A similar experiment was conducted at Rochester, using the varieties Rhode Island Greening and Tompkins King. This work was started on May 21, just as the blossoms were opening. Inoculations above the bags were made on May 22. The results are shown in table 7.

These tests were repeated in 1926 with the same varieties in the same orchards, and in each case the bags were tied over the branches just before the blossoms opened. In addition to the foregoing, thirteen bags were tied on three Wagener apple trees at Ithaca which had blighted severely in 1925. No inoculations were made above these bags, and, as later examination revealed, they were unnecessary, for the majority of the unbagged blossoms, probably 80 per cent, blighted. No blight cankers could be found on the trees at the time when the blossoms were bagged. No blighted blossoms developed in any of the bags on these trees (table 7).

In addition to the foregoing, the writer made observations on apple blossoms inclosed in cheesecloth bags by the Department of Pomology at Cornell University for pollination studies. The varieties used were Rhode Island Greening and Twenty Ounce. About two thousand blossoms were observed in both 1925 and 1926, and, although blossom blight was severe on unprotected blossoms, the blossoms within the bags showed but one cluster blighted. This was on a Twenty Ounce tree and at the extreme end of a branch with no other branches above it. The writer is unable to explain how this cluster became inoculated, unless an insect inoculated it through the bag. No holes could be found in the bag.

From these data it appears that, under the conditions of the experiments, rain at blossoming time did not play a very important part in the dissemination of *B. amylovorus*. It is possible, and highly probable, that where oozing cankers are located above the blossoms, rain may act as a disseminator of fire blight. Instances where this appeared to have happened have been observed, but they could not be verified as hordes of insects had visited the blossoms. The observations of the writer indicate that, as a rule, blossoms do not become sufficiently charged with bacteria to act as centers of inoculation for rain dissemination until after the petals have fallen.

It may be argued that the cheesecloth bags prevented the rain from inoculating the inclosed blossoms. This does not appear probable, as the cloth was of a coarse weave. Since fire blight was severe at Ithaca in both 1925 and 1926, and at Rochester in 1926, disseminators other than meteoric water must have caused the spread.

Field experience in southern Ohio in 1928 adds further evidence of transmission of the fire-blight organism by insects. An isolated apple grower had severe blossom blight on Jonathan and Grimes apple trees in 1927. In 1928 his Jonathan and Grimes trees were free from blossom blight, but his Rome Beauty trees showed from 60 to 80 per cent of blossom infection. No blighted twigs could be found by the writer on the Rome Beauty trees. The Jonathan trees contained many dead twigs from the previous season, and several "hold-over" cankers were found. The explanation appears to be that the two earlier varieties shed their petals before the insects became contaminated, while the Rome Beauty, blossoming later, became inoculated.

Again, in 1930, observations were made bearing on the dissemination of the fire-blight organism in apple orchards of southern Ohio. On April 14, the early varieties had opened about one-fourth of their blossoms and Rome Beauty was in an early pink stage. A week later the petals were falling from the early varieties, and Rome Beauty was in full bloom two days later. Petal fall on Rome Beauty occurred about April 28.

During the period from April 7 to April 28 there was never more than 0.04 inch of precipitation in one day in Lawrence County, Ohio. By May 5, a severe epiphytotic of blossom blight was apparent in Lawrence, Gallia, Meigs, and Athens Counties. In most orchards no active "hold-over" cankers could be found, and yet up to approximately 90 per cent of the blossoms were blighted in extreme cases. No orchard was found entirely free from blossom blight. Comparable conditions were found on twenty-one farms in Brown and Adams Counties on May 19 and 20. The scarcity of sources of inoculum and the virtual absence of rain during the blossoming period, together with the observed incidence of blossom blight, compel the conclusion that in this epiphytotic rain was of no consequence as an agency for the dissemination of the organism. The observed phenomena may be explained, on the other hand, on the assumption that pollinating insects served as the agency for transporting the organism from blossom to blossom and from orchard to orchard.

RELATION OF POLLINATION AND AGE OF BLOSSOMS, TO INFECTION

While some of the earliest workers on dissemination of the fire-blight organism (Arthur in 1885, 1886, and 1887, Waite in 1896, 1898, and 1902, and D. H. Jones in 1909) recognized that open blossoms offer an avenue of entrance for the bacteria, they apparently did not study the susceptibility of blossoms in relation to age or pollination. Gossard and Walton (1922) brought this phase of the problem to our attention. They concluded "that blossoms which have been pollinated for 72 hours are not likely to be inoculated with fire blight and that susceptibility to inoculation does not exist in blossoms that have been pollinated for 144 hours."

The writer, in an effort to test the influence of pollination and age[7] of blossoms on susceptibility to fire blight, started experiments in May, 1925, in two orchards at Ithaca. The trees were of the Tompkins King variety, from thirty to forty years of age, in sod culture, and in fair vigor. Cheesecloth bags were tied over the ends of the branches before blossoming, to prevent pollination of the blossoms by insects. Two series of bags were used. Series I was pollinated as soon as the blossoms opened, and Series II was left unpollinated.

Both series were inoculated by atomizing a bouillon culture of *Bacillus amylovorus* directly into the calyx cup of each blossom when the blossoms had been open for 1 to 5, 48, 72, 96, and 120 hours. The results are given in table 8.

TABLE 8. RESULTS OF BLOSSOM INOCULATIONS, MAY, 1925

Hours from opening to inoculation	Pollinated or not pollinated when opened	Variety	Location	Number of bags	Number of blossoms	Number of blighted blossoms	Per cent blighted	Number of fruits set
1 to 5*	Not pollinated	Tompkins King.......	Ithaca, New York....	2	46	46	100.0	0
48	Not pollinated	Tompkins King.......	Ithaca, New York....	1	22	16	72.7	1
	Pollinated....	Tompkins King.......	Ithaca, New York....	1	21	18	85.7	1
72	Not pollinated	Tompkins King.......	Ithaca, New York....	1	26	26	100.0	0
	Pollinated....	Tompkins King.......	Ithaca, New York....	2	34	30	88.2	2
96	Not pollinated	Tompkins King.......	Ithaca, New York....	1	31	26	83.9	0
	Pollinated....	Tompkins King.......	Ithaca, New York....	2	34	34	100.0	0
120	Not pollinated	Tompkins King.......	Ithaca, New York....	2	42	13	30.9	2
	Pollinated....	Tompkins King.......	Ithaca, New York....	2	46	13	28.3	12
1 to 5†	Not pollinated	Rhode Island Greening	Rochester, New York	3	75	55	73.3	0
48	Not pollinated	Rhode Island Greening	Rochester, New York	2	32	17	53.1	0
	Pollinated....	Rhode Island Greening	Rochester, New York	2	45	10	22.2	0
72	Not pollinated	Rhode Island Greening	Rochester, New York	2	41	20	48.8	1
	Pollinated....	Rhode Island Greening	Rochester, New York	2	46	22	47.8	4
96	Not pollinated	Rhode Island Greening	Rochester, New York	3	87	8	9.2	6
	Pollinated....	Rhode Island Greening	Rochester, New York	3	98	20	20.4	6
120	Not pollinated	Rhode Island Greening	Rochester, New York	3	83	0	0.0	3
	Pollinated....	Rhode Island Greening	Rochester, New York	3	86	0	0.0	14

* Majority of blossoms opened on May 15. A few center blossoms opened on May 14.
† Center blossoms opened on May 22. Remaining blossoms opened on May 25.

The weather during this period was somewhat colder than normal for the season of the year. The maximum and minimum temperatures for 24-hour periods, together with the precipitation, are recorded in table 9.

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[7] "Age," as used by the writer, refers to the time that has elapsed after the opening of the blossom.

TABLE 9. OFFICIAL WEATHER RECORD, MAY, 1925

Location	Ithaca, New York							Rochester, New York										
Date	May 14	May 15	May 16	May 17	May 18	May 19	May 20	May 21	May 22	May 23	May 24	May 25	May 26	May 27	May 28	May 29	May 30	May 31
Precipitation (inches)	Tr.	0	0.03	0.18	0	0	0	0	0.04	0.17	0.04	0	0	0	Tr.	Tr.	0	0.29
Temperature (F.):																		
Maximum	54°	60°	78°	64°	57°	69°	80°	67°	64°	60°	42°	53°	59°	65°	68°	69°	73°	80°
Minimum	42°	35°	34°	42°	35°	33°	42°	45°	44°	36°	32°	40°	40°	42°	51°	54°	53°	55°

The experiment was repeated in an orchard located near Rochester, New York. The variety used was Rhode Island Greening; the trees were about fifty years old, cultivated, and in good vigor. The cheesecloth bags were tied on the branches on May 21, 1925, just as the center blossoms on the south side of the trees were beginning to open. An occasional blossom had opened, and such blossoms were removed before the bag was placed on the branch. The weather on May 22 was warm, and most of the center blossoms unfolded and were cross-pollinated in the afternoon with Baldwin pollen. On May 23 and 24 a cold north wind was blowing, but a little bud development took place. All blossoms were inoculated with bouillon cultures similar to those used in the preceding experiment, mixed with a water suspension of fresh bacterial ooze obtained from Transcendent crab-apple trees.

The results of these two experiments, shown in table 8, indicate that young blossoms are more susceptible to fire blight than are older ones, and that Tompkins King blossoms are more susceptible than are Rhode Island Greening blossoms. Factors other than the variety may have caused the Greening blossoms to blight less frequently than the King blossoms, since the inoculations were made under different weather conditions and in different orchards. Pollination appears to have had little effect on susceptibility to blight, in either the Greening or the King.

In 1926 the experiments were continued in the orchards at Ithaca. On May 28 the center blossoms of the clusters opened and were pollinated with Wagener pollen. The remaining blossoms in the 24-hour series were pollinated in the afternoon of May 29. By May 30 all blossoms had opened and were pollinated. The blossoms were inoculated at the times indicated in table 10. On June 1, when the seventy-two-hour series was inoculated, about 75 per cent of the center blossoms had lost their petals. On June 2, practically all the petals had fallen from the blossoms. The results were tabulated on June 14 and were

TABLE 10. BLOSSOM INOCULATIONS IN RELATION TO AGE AND POLLINATION, MAY TO JUNE, 1926

Hours from opening to inoculation	Pollinated or not pollinated when opened	Date of inoculation	Variety	Location	Number of blossoms	Number of blighted blossoms	Per cent blighted	Number of fruits set
24*	Not pollinated	May 30	Tompkins King.......	Ithaca, New York....	158	137	86.7	9
	Pollinated....	May 30	Tompkins King.......	Ithaca, New York....	198	177	89.4	13
48	Not pollinated	May 31	Tompkins King.......	Ithaca, New York....	298	294	98.7	4
	Pollinated....	May 31	Tompkins King.......	Ithaca, New York....	310	301	97.1	7
72	Not pollinated	June 1	Tompkins King.......	Ithaca, New York....	219	36	16.4	9
	Pollinated....	June 1	Tompkins King.......	Ithaca, New York....	263	33	12.5	73
96	Not pollinated	June 2	Tompkins King.......	Ithaca, New York....	395	24	6.1	8
	Pollinated....	June 2	Tompkins King.......	Ithaca, New York....	390	21	5.4	76
120	Not pollinated	June 3	Tompkins King.......	Ithaca, New York....	450	18	4.0	2
	Pollinated ...	June 3	Tompkins King.......	Ithaca, New York....	336	11	3.3	37
24†	Not pollinated	June 8	Rhode Island Greening.	Rochester, New York.	61	59	96.7	2
	Pollinated....	June 8	Rhode Island Greening.	Rochester, New York.	103	90	87.4	4
48	Not pollinated	June 8	Rhode Island Greening.	Rochester, New York.	99	65	65.6	4
	Pollinated....	June 8	Rhode Island Greening.	Rochester, New York.	116	111	95.7	3
72	Not pollinated	June 10	Rhode Island Greening.	Rochester, New York.	92	48	52.2	6
	Pollinated....	June 10	Rhode Island Greening.	Rochester, New York.	95	85	89.5	5
96	Not pollinated	June 11	Rhode Island Greening.	Rochester, New York.	92	12	13.0	12
	Pollinated....	June 11	Rhode Island Greening.	Rochester, New York.	128	50	39.1	22
120	Not pollinated	June 11	Rhode Island Greening.	Rochester, New York.	147	10	6.8	7
	Pollinated....	June 11	Rhode Island Greening.	Rochester, New York.	149	18	12.1	21
24†	Not pollinated	June 8	Baldwin..............	Rochester, New York.	205	72	35.1	4
	Pollinated....	June 8	Baldwin..............	Rochester, New York.	81	39	48.1	13
48	Not pollinated	June 8	Baldwin..............	Rochester, New York.	253	93	36.7	18
	Pollinated....	June 8	Baldwin..............	Rochester, New York.	132	41	31.1	20
72	Not pollinated	June 10	Baldwin..............	Rochester, New York.	190	26	13.7	13
	Pollinated....	June 10	Baldwin..............	Rochester, New York.	289	40	13.8	31
96	Not pollinated	June 11	Baldwin..............	Rochester, New York.	306	18	5.9	5
	Pollinated....	June 11	Baldwin..............	Rochester, New York.	350	32	9.1	42
120	Not pollinated	June 11	Baldwin..............	Rochester, New York.	220	19	8.6	12
	Pollinated....	June 11	Baldwin..............	Rochester, New York.	163	8	4.9	34

* Center blossoms opened on May 28. Lateral blossoms opened one and two days later.
† Center blossoms opened on June 5 and June 6. Lateral blossoms opened on June 6 and June 7.

rechecked a week later. Owing to the short blossoming period, many of the blossoms in the older series formed abscission layers and fell before the fire-blight organisms from an adjoining blossom could traverse the pedicels and invade them. Thus, in the 24- and the 48-hour series the entire cluster was usually blighted when one blossom became infected, while in the 96- and the 120-hour series there were frequently one or two blighted blossoms in a cluster and the remainder

TABLE 11. OFFICIAL WEATHER RECORD, MAY TO JUNE, 1926

Location	Ithaca, New York													Rochester, New York									
Date	May 25	May 26	May 27	May 28	May 29	May 30	May 31	June 1	June 2	June 3	June 4	June 5	June 6	June 5	June 6	June 7	June 8	June 9	June 10	June 11	June 12	June 13	June 14
Precipitation (inches)	0	0	0	0	0	0.05	0.84	0.26	0.02	Tr.	0	0	0	0	0.01	Tr.	0.01	0	0	0	0.06	Tr	1.41
Temperature (F.):																							
Maximum	62	60°	59°	64°	72°	81°	80°	73°	79°	61°	63°	65°	69°	63°	71°	67°	71	70	73°	75	67°	76°	62°
Minimum	35	39°	36	36°	38°	51	55°	56°	45	43	36°	42	50°	45	50°	54	55°	53°	55°	50°	57	54°	50°

were healthy. The center blossoms blighted as frequently as did the lateral blossoms. Infection sometimes takes place in the blossom, the apple develops, and when the fruit is about one-fourth inch in diameter it is heavily charged with bacteria which ooze from the lenticels during rainy periods. This was observed particularly in the 72- and the 96-hour series.

The test was repeated at Rochester in June, 1926. At the time when the bags were tied about the branches, approximately 50 per cent of the center blossoms of the clusters had opened and these were removed. When the 72-, 96-, and 120-hour series were inoculated, from one-third to four-fifths of the petals had fallen. The results were checked on June 24, 1926, at which time some young fruits one-fourth inch in diameter, and many blossoms, showed drops of bacterial ooze. The results are presented in table 10. The weather record is given in table 11.

The results of 1926 give further evidence of the resistance of blossoms 120 hours after opening, as compared with newly opened blossoms; also, they give no definite evidence that pollinated blossoms are more resistant than unpollinated blossoms to invasion by fire-blight bacteria. The results obtained on the Rhode Island Greening and Baldwin varieties indicate that if pollination does exert an influence on blossoms in relation to fire-blight infection, it is to augment susceptibility rather than to increase resistance. The writer is of the opinion that pollination has very little influence upon infection of blossoms by fire-blight bacteria.

RELATION OF TEMPERATURE TO RATE OF MIGRATION

Most workers on fire blight recognize that temperature is one of the limiting factors in the rate of migration of the bacteria. D. H. Jones (1909) states that the rate of progress of the disease depends largely upon the "succulency of the twig and the atmospheric temperature," warm days inducing rapid progress. Brooks (1926) was one of the first to give particular attention to this phase of the problem. His data (page 688 of reference cited) show that maximum progress was made by the organism at about 25° C.

The writer, in one experiment on the rate of migration (February 22, table 6, page 36), kept a thermographic record during the seven days covered by the experiment. The greatest distances traversed by the organism were recorded on the mornings following the warmest days of the period (February 24 and 27, with a temperature at noon of about 90° F.). Cooler days, although their mean temperature was near 70° F., seemed to be distinctly less favorable for migration. These somewhat fragmentary data are in keeping with the commonly observed fact that fire blight is more destructive in warm than in cool climates.

GENERAL DISCUSSION AND CONCLUSIONS

From a review of the literature and from the writer's work, it is evident that many species of the Rosaceae are attacked by *Bacillus amylovorus.* It is apparent also that species in the genus Pyrus as a whole, are the most congenial hosts for *B. amylovorus.*

Cultural reactions of the fire-blight organism have been determined by various workers. The writer rechecked Stewart's work on acid tolerance and found that *B. amylovorus* can live in an acid medium as low as pH 4.60. Very low temperatures do not kill all of the organisms; some were able to withstand –183° C. for ten minutes in a liquid medium. How long the bacteria would be able to withstand this temperature, or how many repeated freezings would be necessary to give sterile cultures, was not determined.

The toxic principle contained in sap from blighted pear fruit has many of the characteristics of a true bacterial toxin as found in human and animal pathogenes. It will pass through a collodion membrane, is precipitated by alcohol, and is specific for *B. amylovorus*; in contrast with properties of bacterial toxins, it appears to be stable when exposed to the air, and also thermostable. These characteristics suggest that more than one substance may be involved.

It is the opinion of the writer that, when the enzymes are better understood, microchemical tests will reveal some of the reasons for susceptibility or immunity of certain host plants, or of a single host plant at different seasons of the year. At least a knowledge of the usable food of *B. amylovorus* will aid materially in such studies. A parallel exists between phloridzin content of apple shoots, and fire-blight susceptibility. Whether or not the bacteria can utilize phloridzin has not been determined. Sucrase, maltase, inulase, and arbutase were shown to be formed. Amygdalin is found in very small quantities in apple and pear shoots; *B. amylovorus* did not split amygdalin. Arbutin, however, is found in greater quantities in these fruits, and the fire-blight bacteria were found to utilize it. This suggests that at least a part of the food used by the organism may be arbutin. Chemically, arbutin and amygdalin are closely allied. Also, inulin was found to be utilized as a source of carbon, but starch was not.

From the evidence given earlier in this paper, it is apparent that the fire-blight pathogene and some other bacterial plant pathogenes are able to survive adverse conditions for longer periods than was formerly supposed. Just how these organisms can retain their vitality for several years on dead plant parts is not known.

As early as 1885, Arthur pointed out that blossoms are a common avenue of entrance for fire-blight bacteria. From the writer's experience it appears that, in a large proportion of cases, entrance of the bacteria into blossoms is through the nectariferous surfaces. Entrance through stomata or other natural openings was not found in this region of the flower. That the bacteria, after multiplying in the nectar, might gain entrance into the interior through the nectar-secreting cells by either toxic or enzymic action or by both, appears probable. That they can enter a flower also through the petals or the style, has been found by the writer. It is believed that under field conditions the number of infections resulting from the entrance of bacteria through these infection courts is small.

Although some workers report infection through either stomata or hydathodes, the writer was unable to consistently obtain infection through these openings. Artificial inoculation on uninjured apple and pear seedlings, indoors and out, failed to give consistent evidence of such a mode of ingress. Infection of pear and apple leaves can be readily obtained through needle or insect punctures. It has been the writer's experience that such inoculations rarely produce infection of the twig, as the bacteria usually die before reaching the base of the petiole.

After the bacteria have been introduced into the interior of young, rapidly growing, apple shoots, there appears to be a delay before much new tissue is invaded. No instances were found in which the bacteria progressed as much as an inch during the first day after inoculation. Beginning twenty-four hours after inoculation, the rate of progress, on an average, was little over 1 inch a day. No cases of 8 to 10 inches of migration a day, as reported by some workers, were found, although an attempt was made to maintain optimum conditions for migration.

For a number of years, insects were considered the chief disseminating agents of fire blight. In 1922, Gossard and Walton again placed this phase of the problem in question. That insects are the principal vectors of the fire-blight germs under certain conditions can hardly be contested, in view of the careful work done by a number of workers. The writer's experiments and field observations bearing on this question, point strongly to insect transmission from blossom to blossom. But how are the first blossoms inoculated? If oozing cankers occur immediately above open blossoms, there is no apparent reason why meteoric water cannot inoculate them, since blossoms are easily inoculated by atomizing. However, the number of blossoms so inoculated would be relatively small when all cankers and dead twigs are carefully excised.

Gossard and Walton have held that pollinated blossoms are less subject to infection than are unpollinated blossoms. The data given in this paper show that increasing *age* of blossoms, and not *pollination*, reduces the susceptibility to fire-blight infection. It is a well-established fact that the blight pathogene thrives best in rapidly growing tissue. On this basis one might expect pollinated blossoms which are actively growing to be more subject to attack than unpollinated ones. There is some evidence in this paper in support of this hypothesis.

SUMMARY

Additional information is presented in this paper on the susceptibility of rose, strawberry, and *Crataegus oxyacantha*. Japanese quince (*Chaenomeles lagenaria*) and *Photonia villosa* are added to the list of suscepts.

It is held that *Bacillus amylovorus* can live in an acid medium of pH 4.60, that it can withstand a temperature of -183° C. for ten minutes, and that heating to 48° C. for ten minutes does not always inhibit growth.

Positive evidence is given for the formation of the enzymes sucrase (or invertase), maltase, inulase, and arbutase by *B. amylovorus*; negative results are reported for amylase, pectosinase, pectase, pectinase, cellulase, amygdalase, and trypsin.

A toxic substance was found in green pear fruits which were rotted by the fire-blight organism.

Viable bacteria were isolated from ''hold-over'' cankers on twigs as small as one-fourth inch in diameter.

Pathogenic bacteria were obtained from infected limbs after these had been kept in the laboratory for two years.

The commonest mode of entrance into blossoms was found to be through the nectariferous surfaces; occasional entrance from natural and artificial inoculation was found to occur through the petals and the styles.

Naturally inoculated pear leaves were found on trees infested with leaf hoppers. Artificial inoculation by atomizing young, rapidly growing, pear and apple trees failed to produce infection if the trees were free from insects.

B. amylovorus was found to progress slowly for the first twenty-four hours after inoculation into young apple shoots; thereafter, for a period of eight days, migration was found to proceed at the rate of about 1 inch a day.

Meteoric water did not appear to spread the blight bacteria from blossom to blossom.

Increasing age of blossoms was shown to decrease susceptibility to fire blight. Pollination of blossoms was found to have little, if any, influence on susceptibility.

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AGRICULTURAL EXPERIMENT STATION

STUDIES ON THE DOWNY MILDEW OF ONIONS, AND THE CAUSAL ORGANISM, PERONOSPORA DESTRUCTOR (BERK.) CASPARY

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STUDIES ON THE DOWNY MILDEW OF ONIONS, AND THE CAUSAL ORGANISM, PERONOSPORA DESTRUCTOR (BERK.) CASPARY[1]

Harold Thurston Cook

Downy mildew of the onion has been known for many years and frequently has been a subject of study. In spite of this, our knowledge of the life history of the pathogene and its environmental relations is still incomplete. The disease continues to be one of the most destructive and widespread diseases of the onion, since, for the most part, the measures recommended for its control have been impracticable and have yielded uncertain results. With heavy losses occurring in the vicinity of Elba, in western New York, during the summers of 1926 to 1929 while the writer was engaged in investigating diseases of muck crops,[2] a study of the disease and the pathogene was undertaken. The results of the investigation, together with a review of results reported by other workers, are here set forth.

NOMENCLATURE OF THE CAUSAL ORGANISM

Although the fungus causing downy mildew of onions was placed in its proper genus soon after its discovery, various specific names have been given to it. A chronological review of the taxonomy of the onion-mildew organism, and the writer's conclusions regarding the valid name, follow.

So far as is known, *Botrytis destructor* is the first name applied to the pathogene. It was published in 1841 by Berkeley, with a Latin description and an illustration of the conidial stage of the fungus. The perfect stage was not included in this description.

The name *Botrytis* (*parasitica?*) is used by Schleiden in connection with a description and a figure of the fungus in *Grundzüge der Wis-*

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[2] Acknowledgment is made of the cooperation of members of the Genesee-Orleans Vegetable Growers' Association, whose support of a special temporary fellowship made these studies possible.

senschaftlichen Botanik. There is some disagreement in regard to the date when this name was first published. Whetzel (1904) gives the third edition of Schleiden's work (1850) as the first place and date of publication; but Unger (1847), three years before the third edition was published, cited this name. Although the writer has been unable to consult the second German edition, which was published in 1845–46, the fact that this name is in the English translation of that edition but not in the first German edition, both of which have been examined, indicates that it was first published in the second German edition in 1845–46. This is probably merely a case of mistaken identification, and therefore has no standing in synonymy. The form in which Schleiden wrote the specific name (in parentheses with a question mark) and the fact that this name has already been applied by Persoon in 1796 to another fungus, occurring on the Cruciferae, indicate Schleiden's uncertainty.

Peronospora schleideni is given by Unger, with a short Latin description of the conidial stage, in 1847. Since no mention of the perfect stage is made in Unger's paper, the name is not valid according to the International Code of Nomenclature, and, furthermore, the specific name of the imperfect stage is antedated by *Botrytis destructor* Berkeley (1841).

Peronospora destructor Caspary is the name under which the fungus was listed by Berkeley in 1860, and reference was made to an earlier description of the fungus by him (1841) under the name *Botrytis destructor*. The fact that, in the generic description of Peronospora, Berkeley states that this genus possesses oospores, and explains in a footnote that this generic name was adopted because of the discovery of oospores, indicates that the perfect stage of the fungus was known at that time. The writer has been unable to determine Berkeley's reason for attributing this name to Caspary. Wilson (1914) suggested that it was probably in recognition of some manuscript name. Caspary (1855) did publish, however, a paper in which he described the discovery of oospores in *Peronospora parasitica* and other species of this genus. He puts the onion-mildew fungus in the list of those species which he says were seen either in the imperfect stage or not at all ("Species generis Peronosporae, quas vel tantum in statu manco vel non vidi"). In listing this fungus he calls it *Peronospora schleideni*. Possibly in a later paper he announced the discovery of oospores in the onion-mildew fungus, and used the new combination which Berkeley attributed to him.

De Bary used *Peronospora schleideniana* Unger in listing and describing this species in his revision of the genus Peronospora in 1863. He gave no reason for changing the form of the specific name as it was originally published by Unger. Farlow (1884) suggested that the change was made on etymological grounds, while Gäumann (1923) said that it probably was an error on the part of de Bary.

Peronospora alliorum is the name under which this fungus was distributed by Fuckel in 1863 in Fungi rhenani exsiccati no. 41. Later Fuckel (1869) accepted the name given by de Bary and placed *Peronospora alliorum* in synonymy under *Peronospora schleideniana*.

As a result of de Bary's influence the fungus was known under the name of *Peronospora schleideniana* for many years, but recently *Peronospora schleideni* has been used more commonly.

Wilson (1914) discussed the synonymy of this fungus briefly, and considered *Peronospora destructor* (Berk.) Caspary the correct name. Gäumann (1923), however, disagreed with Wilson on this point, and insisted that the correct name is *Peronospora schleideni* Unger, since this antedates *Peronospora destructor* by thirteen years.

Gäumann was correct as far as the dates of publication of the binomials are concerned, but he evidently overlooked the fact that the species name "*destructor*" antedates "*schleideni*" by six years, and that the perfect stage is included in the description of *Peronospora destructor* while only the conidial stage is described for *Peronospora schleideni*.

The writer concludes that the valid name of the causal organism of downy mildew of onions is *Peronospora destructor* (Berkeley) Caspary, with synonymy as follows:

Botrytis destructor Berkeley, Ann. and Mag. Nat. Hist. **6**:436. 1841.
Peronospora schleideni Unger, Bot. Ztg. **5**:315. 1847.
Peronospora schleideniana (Unger) de Bary, Ann. Sci. Nat. 4:**20**:122. 1863.
Peronospora alliorum Fuckel, Fung. rhen. n. 41. 1863.

DISTRIBUTION

Downy mildew is present, and often destructive, on onions of various kinds in nearly all parts of the world. It is known to occur in Bermuda, the Canary Islands, China, Denmark, England, France, Germany, Holland, Ireland, Italy, Japan, Mauritius, New Zealand, Norway, Russia, Spain, the United States, and Western Australia. In the United States it has been reported in the *Plant Disease Reporter* (1917 to 1930) from the following States: Arkansas, California, Colorado, Connecticut, Georgia, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, New York, North Dakota, Ohio, Oregon, Pennsylvania, Vermont, Washington, West Virginia, and Wisconsin. The geographical distribution of the disease in this country, and the number of times that it has been reported from each State, in the *Plant Disease Reporter,* are shown in figure 1. Apparently it has been observed most frequently in New York, California, Oregon, Louisiana, and Ohio. Its wide distribution, however, suggests that it may occur commonly and in other States than those shown by the reports. Probably the reason why it has not been reported from more localities is that the crop or the disease is not of sufficient economic importance to attract attention.

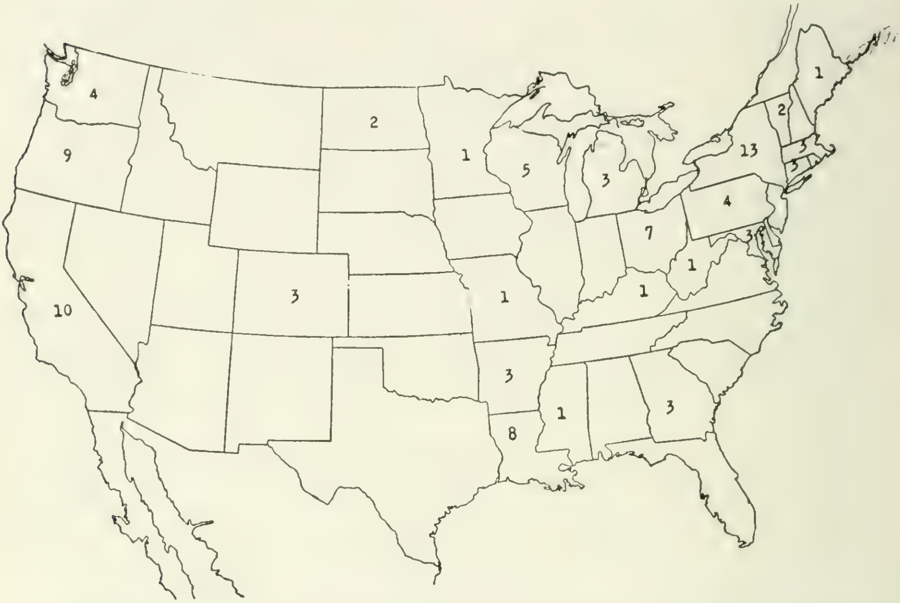

FIGURE 1. GEOGRAPHICAL DISTRIBUTION OF ONION MILDEW IN THE UNITED STATES

The numerals show the number of times that the disease has been reported in the *Plant Disease Reporter,* for each State

HISTORY

The first report of onion mildew was made by Berkeley in England in 1841. Although the disease was frequently reported during the next forty-three years in Europe (Schleiden 1842, Unger 1847, de Bary 1863, Fuckel 1869, Frank 1880, and Smith 1884), it was not until the paper by Shipley (1887) appeared that a real contribution to our knowledge of the disease was made. Shipley was appointed by the British Government to investigate the cause of the heavy losses to the onion crop in Bermuda. He began his study by visiting the Canary Islands, where the onion seed used in Bermuda was grown, and found onion mildew to be one of the most serious onion diseases there. He made careful observations of the effect of the disease on the plants, and of the environmental conditions favoring it. In the following year he investigated the disease in Bermuda, where his earlier observations were confirmed.

Onion mildew was first reported in America by Trelease (1884). In the first annual report of the Wisconsin Agricultural Experiment Station, he devoted several pages to a discussion of the disease, which apparently was important in the Middle West at that time. Shortly afterward, Dudley (1889) found that onion mildew was destructive in New York, and Thaxter (1890) reported it on seed onions in Connecticut. L. R. Jones (1896) found it very destructive in Vermont. Whetzel (1904) gave the most complete account of the disease that had been published up to that time. No other work of consequence relating to onion mildew appeared for the next seventeen years. It is strange that such an important disease should have been so completely neglected during this period, when plant-pathological activities were expanding rapidly.

Beginning with the publication of the *Plant Disease Reporter* in 1917, there have been numerous reports of the seriousness of this disease in various parts of the country. It has been present to some extent every year, and in some years serious losses were caused by it.

More recently Murphy (1921) and Murphy and M'Kay (1926) in Ireland, Katterfeld (1926) in Russia, and Hiura (1930, a and b) in Japan, have added materially to our knowledge of onion mildew.

SUSCEPTS

Peronospora destructor appears to be confined to the genus Allium. It has been reported in literature on the following species:[3]

Allium ascalonicum Linn. (Shallot). Ritzema Bos (1898).
Allium cepa Linn. (Common onion). De Bary (1863).
Allium cepa var. *bulbellifera* Bailey (Egyptian or tree onion). Murphy and M'Kay (1926).
Allium cepa var. *multiplicans* Bailey (Potato or multiplier onion). Murphy and M'Kay (1926).
Allium fistulosum Linn. (Welsh onion). Schleiden (1842).
Allium porrum Linn. (Leek). Schöyen (1901).
Allium sativum Linn. (Garlic). Caballero (1922).

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[3] The scientific names of the suscepts are taken from *The Standard Cyclopedia of Horticulture,* 1922, by L. H. Bailey.

Sorauer (1886) and Tubeuf (1895) stated that wild species of Allium are attacked by mildew, but did not give the specific names of the suscepts.

Cross-inoculation experiments were conducted, in the course of these investigations, on four species and varieties of Allium and one species of a closely related genus. The inoculum used in these experiments was obtained from *Allium cepa* var. *bulbellifera.* In all cases the plants were repeatedly inoculated under conditions favoring infection. Infection was obtained on *Allium cepa* and on *Allium schoenoprasum* Linn. So far as is known, this is the first record of the disease on *Allium schoenoprasum,* and specimens have been deposited in the herbarium of the Department of Plant Pathology at Cornell University.

Allium porrum, Allium sativum, and *Nothoscordum bivalve* Brit. (yellow false garlic) did not become diseased when inoculated, although susceptible species inoculated at the same time and under the same conditions did. Field observations failed to reveal the fungus on *Allium tricoccum* Ait. (wild leek), which grows near the onion fields at Elba. *Allium vineale* Linn. (field garlic) also failed to become infected, although it was planted in one of the experimental plots in which the disease was prevalent.

VARIETAL SUSCEPTIBILITY

Very few investigations in regard to varietal susceptibility of onions to mildew have been reported in literature. Shipley (1887) reported that both red and white varieties are attacked, but that the red varieties are the more resistant. Rosa (1926) observed that in California the seed-stalks of the white varieties are attacked earliest and most severely, and H. A. Jones (1926: 69) stated: ''The foreign types like Giant Gibraltar, Sweet Spanish, Prizetaker, etc., with light green tops, appear to be somewhat more resistant than the storage varieties like Yellow Globe, Danvers, and Southport.'' Murphy and M'Kay (1926) reported differences in the susceptibility of the varieties under their observation. They based their conclusions on the percentage of dead plants, but stated that at the time when the counts were made practically all of the plants were diseased. Murphy and M'Kay list the nineteen varieties under their observation in the order of their resistance.

Fifty-three varieties of the common onion were grown and examined under field conditions for differences in susceptibility, in the course of this study, but practically 100 per cent of the plants became infected. Striking differences in the number of plants with dead and with living tops appeared to be due to early- or late-maturing characteristics of the varieties, rather than to the mildew. The varieties tested are listed in table 1.

TABLE 1. VARIETIES OF THE COMMON ONION TESTED FOR SUSCEPTIBILITY TO PERONOSPORA DESTRUCTOR*

1. Ailsa Craig
2. Australian Brown
3. Autumn Queen
4. Bedfordshire Champion
5. Blood Red
6. Bronze Globe
7. Ciboule Blanch Native
8. Crystal White Wax
9. Dark Red Brunswick
10. Earliest White Queen
11. Early Danvers
12. Early Large Red
13. Ebenezer
14. Extra Early Pearl
15. Extra Early Red
16. Froxfield
17. Giant Gibraltar
18. Giant Rocca
19. Golden Globe Tripoli
20. Holborn
21. Iwatsuki
22. James Long Keeping
23. Large Red Italian
24. Michigan Yellow Globe
25. Mountain Danvers
26. Mountain Red Globe
27. Natsu-negi Nebuka
28. New Mammoth Silver King
29. Nuneham Park
30. Ohio Yellow Globe
31. Pear-Shaped
32. Pearl Pickler
33. Prizetaker
34. Record
35. Red Wethersfield
36. Round Yellow Danvers
37. Rousham Park Hero
38. Senju-negi Nebuka
39. Southport Red Globe
40. Southport White Globe
41. Southport Yellow Globe
42. Straw-colored Flat Keeping
43. "The Perfect" Long Keeper
44. Tokyo Nebuka
45. Trebons
46. Up-to-Date
47. White Portugal
48. Winterheck
49. White Welsh
50. White Emperor
51. Yellow Bermuda
52. Yellow Danvers
53. Yellow Globe Danvers

* Some of these observations were made on varieties grown by E. L. Felix in testing for resistance to other diseases.

IMPORTANCE AND NATURE OF THE LOSSES

The frequent and heavy losses caused by the onion mildew have been reported by numerous writers from the time when the disease was first recorded by Berkeley (1841). Many investigators have stated that the disease causes serious damage to all stages of the common onion and other species of Allium. The actual reduction in yield is sometimes as high as 60 to 75 per cent of the normal crop.

The onion plant may be attacked and destroyed in the seedling stage, as has occurred several times in Georgia (Boyd, 1925, and Higgins, 1925). In greenhouse experiments it was found that infected onion seedlings usually died soon after the fungus fruited. Possibly some of the damping-off of the seedlings is attributable to mildew instead of to Botrytis, to which it usually is attributed.

When older plants are attacked by mildew, the bulbs are reduced in size. This fact was noted in the first written account (Berkeley, 1841), in which it was stated that the disease prevents the onions from coming to perfection. Whetzel (1904) discussed this point in considerable detail, emphasizing the fact that the fungus destroys the leaves at the time when they should be manufacturing food for the bulb, which then ceases to grow while new leaves are being developed. In turn these new leaves may be destroyed by the fungus soon after they are formed, thus further retarding the development of the bulbs. Whetzel stated that there is little or no increase in size of the bulbs following a severe attack of the fungus.

Observations by the writer also indicated that the bulbs of mildewed plants are considerably reduced in size, and an experiment was conducted in 1927 to obtain more definite information on this point. On August 4, approximately ten days after the mildew was found in the field, the circumference of the bulbs of twelve diseased and twelve healthy plants was measured. This was accomplished without disturbing the plants, by carefully scraping a little of the soil away from the bulbs, which were already half exposed, a natural condition on muck soil. The same bulbs were measured a second time on September 1, four weeks later. Unfortunately, by that time even the plants which were healthy at the first measuring had become diseased, and so it was possible only to compare early-infected plants with those which became infected late. The results of these measurements are given in table 2.

TABLE 2. EFFECT OF DOWNY MILDEW OF ONIONS ON INCREASE IN SIZE OF THE BULBS*

Early-infected plants			Late-infected plants		
Bulb no.	Circumference in inches		Bulb no.	Circumference in inches	
	August 4	September 1		August 4	September 1
1	6.00	6.25	1	6.00	7.25
2	6.00	6.25	2	6.00	9.25
3	6.25	6.75	3	6.25	8.50
4	7.25	7.75	4	7.25	9.00
5	6.25	6.50	5	6.75	8.75
6	4.75	5.50	6	7.00	9.00
7	6.25	7.00	7	7.75	9.00
8	5.25	5.75	8	7.00	8.50
9	5.75	6.00	9	5.50	7.00
10	5.75	6.25	10	5.00	7.75
11	5.75	6.50	11	6.00	7.25
12	6.50	7.25	12†	....	....
Average	5.979	6.479	Average	6.409	8.295
Average increase in circumference 0.5 ±0.04			Average increase in circumference 1.886 ±0.14		

The increase in the circumference of the bulbs of late-infected plants over that of early-infected plants is 1.386 ±0.15. The odds that the difference is significant are greater than 9999 to 1.

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* The probable error was calculated by Bessel's formula, P. E. $= \frac{0.6745 \times \sigma}{\sqrt{n-1}}$. This formula may not give the true probable error, since the average size of the bulbs in the late-infected group was slightly larger on August 4 than that of the early-infected group. Therefore the probable error of the first four pairs in the table, which were of the same size on August 4, was calculated according to Student's method for interpreting paired experiments described by Love and Brunson (1924). By this method the mean difference of the increase in size of the bulbs of the late-infected plants over that of the early-infected plants is 1.75 inches. The standard of deviation is 0.77, and the odds that the difference is significant are 65.2 to 1.

† This plant was missing on September 1.

The figures given in table 2 show that there is a very marked difference in the rate of growth between the bulbs of plants infected early and those of plants infected late. It is reasonable to suppose that the difference would have been even greater if the bulbs of the diseased plants had been measured earlier and the healthy ones had not become infected.

Onion mildew causes losses to the crop in storage as well as in the field. Murphy and M'Kay (1926) found that bulbs containing perennial mycelium of the onion-mildew fungus produced green shoots prematurely and then turned soft and rotted. These writers found that such losses were common in the very susceptible varieties. The present writer has noticed that in years when mildew was severe, many thick-necked onions were produced. Such onions do not cure properly, and rot in storage.

Seed plants also may be attacked. In such cases the stalks are either killed outright, causing a total loss of the seed, or they are weakened to such an extent that the seed which is formed is of inferior quality. Also, there are strong indications that the seed obtained from diseased plants may be infected or infested and thereby introduce the disease into the next crop.

FIELD OBSERVATIONS ON THE OCCURRENCE OF ONION MILDEW

SIGNS AND SYMPTOMS

The first signs of onion mildew on early-infected plants is the production of conidiophores a short distance back from the tips of the older leaves. Murphy and M'Kay (1926) considered this a sign of systemic infection. The conidiophores have a purplish tinge when fresh, and give the lesions a downy appearance. Soon the affected part of the leaf turns yellow, withers, and breaks over. Afterward the fungus may fruit at any point on the leaves with the same ultimate result, the death of the affected tissues. The writer's observations do not confirm Murphy and M'Kay's report, that the formation of the conidiophores is preceded by a pale yellowing of the affected parts of the leaves. Instead the yellowing was found to follow closely after the formation of the fungus fruiting structures.

The fungus may fruit on any part of the seed plants, but the fruiting structures usually appear first on the leaves and then on the seed-stalks. They may occur at any point on the seed-stalk from the base to the top, and occasionally are found on the infloresence. Shortly after the fungus has fruited, the epidermal and palisade cells of the affected parts collapse and the resulting lesion becomes white, slightly sunken, and roughened. The lesions are usually circular or elliptical in shape, and involve only one side of the stalk. In some cases, however, they are large and girdle the stalk. Lesions very similar in appearance often result from mechanical injury, such as is caused by two stalks rubbing together. Such lesions have been called the "white spot disease" by Edgerton (1921), who, finding that the tissues were sterile at first, suggested that they were probably physiological in nature. In wet weather, both the mildew lesions and those caused by mechanical injuries are soon invaded and overrun by Macrosporium. The lesions then become brownish black in color. This stage is called by Edgerton the "black stalk rot disease." The mildew lesions are so similar to the white-spot lesions that the only reliable way of distinguishing between them is by microscopical examination.

The effect of the disease on the production of seed depends on the time when infection takes place, the point at which the lesion develops, and the rate of spread of the fungus throughout the plant. Early infections usually cause greater damage than do late ones, since they weaken the plant before the seeds have had an opportunity to develop, and there is also more time for the fungus to spread throughout the plant. However, the point of attack and the nature of the lesion formed have much to do with the damage to the plant. Lesions on the lower half of the stalk, especially those that girdle it, cause the stalk to fall over, and the infloresence, being in contact with the moist ground, may be attacked by Botrytis. Lesions that occur on the upper half and rather late in the season, however, permit seed to be formed although the stalk is completely girdled. Such lesions seldom cause the stalk to break over, and sufficient nutrients remain in the tissues above the lesions to supply the needs of the maturing seed.

The signs and symptoms of the disease on set onions and on Egyptian, or top, onions are similar to those on the seed plants and on market onions.

TIME OF OCCURRENCE

Under New York conditions, mildew may be found in the field on market onions from the middle of July to the end of the season. In 1926, at Elba, New York, the first mildewed plants were discovered on August 4. At that time, however, the wide distribution of the disease, and the advanced symptoms on many of the plants, indicated that the disease had been present for some time. In the following year more careful observations were made and the first diseased plants were found on July 23, but even then it was evident that the mildew had been overlooked for some time. During the next two years the results of the observations were similar. Although the onion fields were inspected carefully throughout the season, the first diseased plants were never found earlier than July 23, but each time evidence indicated that the mildew had been present for some time previous to its discovery. The disease is probably present to some degree from the seedling stage. Owing to the small number of diseased plants and the fact that the fungus may not fruit very abundantly because of unfavorable environmental conditions existing early in the summer, mildew spreads very slowly at that time and is not noticed. By July or August, however, more plants have become infected, and with favorable weather conditions the disease develops and spreads very rapidly.

Mildew usually is found about two weeks earlier on set and seed plants than on market onions. In 1928 the disease was first observed by the writer on set onions on July 10, and the grower on whose farm it was found stated that it had already been present for two weeks. It was not found, however, on market onions growing in the next row until July 20. By that time it was present also on market onions in all other fields about Elba, indicating that there were sources of inoculum other than the fungus on set onions.

Mildew was found on Egyptian, or top, onions as early as the first week in July in some seasons, and was observed fruiting on them in one planting at Ithaca as late as November. The fungus seems to be perennial in these plants, and fruits whenever favorable conditions exist.

Experience has shown that a few diseased plants always can be found, even in seasons when the disease is considered to be absent. Consequently, when favorable weather conditions exist an epiphytotic may occur.

LOCATION OF DISEASED PLANTS

Each season it was observed that the disease occurred at first on individual plants which were scattered uniformly throughout the fields. Furthermore, during this early period mildew was as prevalent in fields where onions had never been grown as on old onion land. Nor did there seem to be any difference in the occurrence of the disease in fields near plantings of sets and seed onions and in fields that were isolated. After a few days of weather favoring the development and spread of the disease, the mildew became more severe on the plants next to the hedges, in low spots, where the top growth was heavy, and in other sheltered situations. It is observations on these latter stages of the disease that have given rise to the frequent erroneous statements that the disease starts in certain sheltered parts of the field and spreads to the remainder. The mildew at first is distributed uniformly throughout the field, but it spreads more rapidly in certain parts of the field than in others.

TEMPERATURE AND MOISTURE RELATIONS

Moisture was observed to have a great influence on the development and spread of the onion-mildew fungus. The formation of the fruiting bodies appears to be dependent, for the most part, on an abundance of moisture. The conidia are produced in greatest numbers during rainy periods and when the leaves are wet with dew. They are never produced during dry periods when the leaves are entirely free from moisture. An examination of the thermograph records which were kept during the course of this investigation showed that the fungus fruits at a time of the year when the night temperatures vary between 5° and 13° C.

The effect of temperature and moisture on the viability of the conidia was even more marked. In obtaining spores for germination tests and inoculation purposes, it was found necessary to collect them early in the morning, before the dew had dried or the spores had been exposed to the direct rays of the sun. Desiccation and heat are fatal to the conidia.

With market onions it was observed that the mildew spreads most rapidly where the foliage is heavy, near hedges, in low spots, and in fields that are sheltered from the sun and wind. These conditions favor the accumulation and retention of water on the foliage of the plants, and thereby make it possible for the fungus to fruit and to infect other plants. Two seed plots on one farm, which were observed in 1927, are good illustrations of the effect of environmental conditions on the occurrence of onion mildew. One plot was located in a field that was bounded on the south by an orchard and on the west by a large barn. The planting was poorly cultivated and consequently many weeds were present. The protection thus afforded the plants prevented the rapid drying of the leaves. In this plot 85 per cent of the plants became diseased. The other plot was kept free of weeds, and, being situated on the west side of the barn, was more exposed to the prevailing winds, which favored drying of the leaves. Only 15 per cent of the plants in this field were mildewed. Another seed plot which was observed in that year was located on muck soil where the humidity was high. That also was allowed to be overgrown with weeds, and practically every onion plant became diseased.

SOURCES OF PRIMARY INOCULUM

It was deemed necessary to determine the principal sources of the primary inoculum so that methods could be developed to eliminate them and thus prevent the disease from gaining a foothold in the field. This conclusion was reached when attempts to combat the disease after it had appeared in the field resulted in failure. Even if it were possible to control the disease after it had appeared, the injury suffered by the plants from the initial attack would often be so great that they would not recover sufficiently to produce a full crop. Therefore the evidence in support of four theories as to sources of primary inoculum was considered. These possible sources were: (1) systemically infected plants; (2) infested soil; (3) infested seed; and (4) infected seed.

SYSTEMICALLY INFECTED PLANTS

Murphy (1921), Murphy and M'Kay (1926), and Katterfeld (1926) reported that a large number (approximately 45 per cent) of the onion bulbs from fields in which the disease was severe were systemically infected with perennial mycelium of the mildew fungus. They presented evidence that this perennial mycelium is one of the principal sources of primary inoculum where onions are sown in the fall and allowed to overwinter in the field. Since this cultural practice is not followed at Elba, fall-grown onions could not be a source of primary inoculum in this section.

Perennial mycelium in onion sets, seed plants, and volunteer onions may be a source of a part of the primary inoculum in the area where these investigations were made. This is suggested by the fact that the fungus fruits on sets and seed plants several weeks in advance of its appearance on market onions. Mildewed sets and seed onions cannot be considered the main source, however, in the Elba section because there are comparatively few plantings of these on the muck, and the seedlings become diseased as early in the fields at a distance from the sets and the seed onions as in those near by. Although no volunteer onions have been observed to be infected, it is conceivable that some of them are diseased and furnish a part of the primary inoculum.

Tubeuf (1895) stated that the onion-mildew fungus occurs on wild species of Allium, but did not mention the specific names of the suscepts. The only wild species of this genus at Elba are wild leeks; and, since these were never observed to have mildew, it is very doubtful whether they are of any importance as a source of primary inoculum.

INFESTED SOIL

Oospores in the soil are probably one of the sources of primary inoculum. Although Dudley (1889), Murphy and M'Kay (1926), and many other writers on onion mildew, stated that oospores are rarely formed, Shipley (1887), L. R. Jones (1896, 1897), Whetzel (1904), Katterfeld (1926), and Hiura (1930 a) reported having found them in relatively large numbers. Jones (1897) determined experimentally that plants grown on oospore-infested soil which had overwintered became diseased, while those on clean soil did not. In spite of the lack of agreement on the importance of oospores, they have been observed in large quantities by a sufficient number of capable workers to establish their importance as a means of propagating the fungus.

Conidia are found in the soil, but, since they are very short-lived, it is unlikely that they are of importance in producing infection. Under the most favorable conditions conidia remain viable for only a few hours, and almost certainly do not function in carrying the fungus over winter.

Mycelium in the soil is a possible, but not probable, means by which the fungus overwinters. No evidence in support of this theory is known.

INFESTED SEED

Oospore-infested onion seed may be a source of inoculum. Chapman (1910) reported the presence of spores of *Peronospora destructor* with onion seed, but did not specify whether he found oospores or conidia. Chupp (1925) states: ''An examination of the seed has revealed an admixture of oospores.'' If this statement is based on Chapman's paper it is erroneous, since the kind of spores found was not specified. Murphy and M'Kay (1926) also assumed that Chapman meant oospores. Katterfeld (1926) found that oospores occur in the pedicels of the flowers, and Hiura (1930 a) reported that oospores are occasionally found in the flower stalks and the capsules. This being the case, it is easily understood how the oospores might become mixed with seed during the threshing process. L. D. Leach[4] stated that oospores are present in large numbers in California. This fact is especially significant, since California is the largest onion-seed-producing center in the world.

In order to determine definitely whether or not oospores do occur with the onion seed, a number of samples of commercial seed were examined by the writer. This was done by shaking a quantity of seed with about twice its volume of water, which was then poured off, centrifuged, and the sediment examined for the presence of spores. A few oospores were found in several of the samples.

The behavior of the disease at Elba suggests dissemination of the mildew fungus with the seed, since, as was shown by field observations, the disease occurred on scattered individual plants in new and isolated onion fields, which is characteristic of seed transmission. Such dissemination may occur partly by oospores mixed with the seed.

Conidia mixed with the seed are a possible, but not probable, source of primary inoculum. Since they are very sensitive to drying, they would not be able to survive storage with seed.

INFECTED SEED

A considerable amount of evidence was found in support of the theory that infected seed may be one source of primary inoculum. The distribution of the disease early in the season, which is characteristic of seed transmission, has already been mentioned in the discussion of infested seed. Such distribution may also indicate infected seed. Another reason for thinking that infected seed may have a part in the spread of onion mildew is that seed plants are frequently attacked by the mildew. This was observed at Elba, New York, and onion mildew has been reported nearly every year as causing heavy losses in the onion-seed-producing sections of California.

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[4] In a letter dated August 5, 1930.

In order to determine whether or not seed infection actually occurs, close attention was given to the plantings of seed onions. Although seed are not produced commercially at Elba, a few are grown each year by some of the growers for their own use.

The seed plots were closely observed and many flowers and seed were sectioned, but it was not until 1928 that proof of floral infection was obtained. At that time a single seed-stalk having an infected inflorescence was found. This stalk had a mildew lesion just below the flower umbel, and the fungus was fruiting sparsely on some of the flower pedicels. Microscopic examination of the conidiophores showed the fungus to be *Peronospora destructor*. Sections of the seed-stalk, the

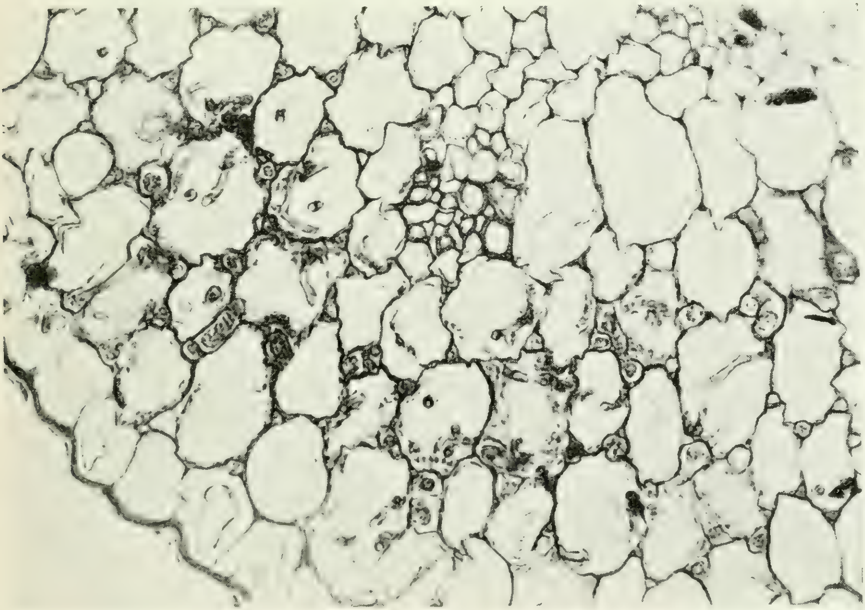

Figure 2. cross section of a pedicel of an infected onion flower, showing the intercellular hyphae and the intracellular haustoria of the fungus

pedicels, and the flowers, prepared at the field laboratory with a freezing microtome and stained with cotton blue in lactophenol or with one-half per cent eosin in water, demonstrated the presence of mycelium, which could be traced from the stalk up through the pedicel into the base of the ovary. In some sections conidiophores were observed arising from the mycelium in the pedicels, thus proving the identity of the fungus. The remainder of the infloresceuse was fixed in chrom-acetic- and picric-acid fixing solutions, dehydrated, and embedded in paraffin for later study. Sections of this material, cut 7 microns thick and stained with Haidenhain's haematoxylin, Flemming's triple, or Delafield's haematoxylin, demonstrated the extent of the floral invasion by the mildew fungus. These sections showed that all of the flower parts had been invaded. There was an abundance of the mycelium in the pedicels (figure 2), where it consisted largely of long, straight

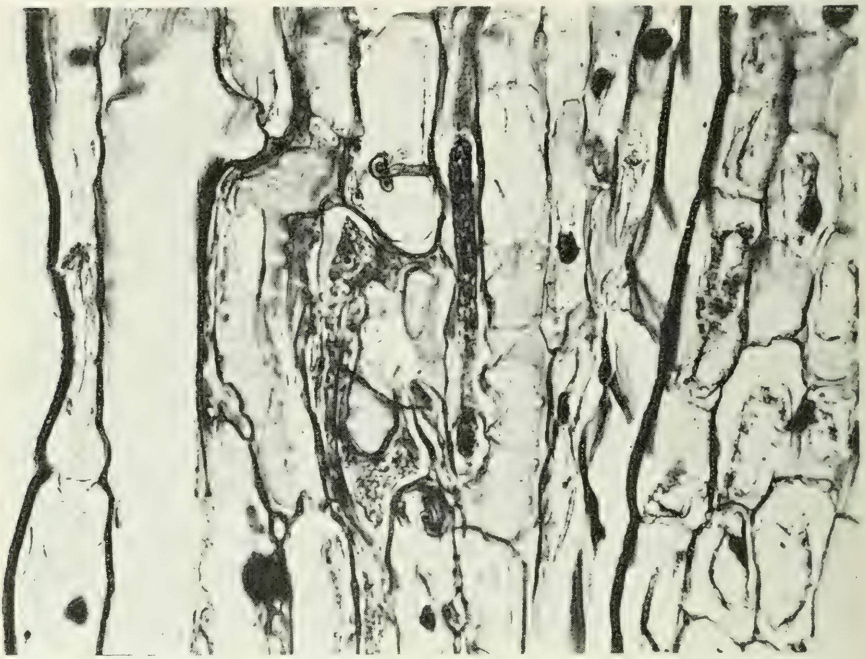

FIGURE 3. LONGITUDINAL SECTION OF A PETAL OF AN INFECTED ONION FLOWER, SHOWING THE LONG, STRAIGHT, INTERCELLULAR HYPHAE AND THE INTRACELLULAR HAUSTORIA OF THE FUNGUS

hyphae with numerous long and branched haustoria penetrating the cells. The mycelium itself was always intercellular, but the size and number of haustoria sometimes gave the appearance of the fungus being intracellular. The mycelium was irregular in width, depending largely on the size of the intercellular spaces through which it was passing. The fungus was easily traced from the pedicel into the receptacle, where it branched in all directions and progressed toward all of the flower organs. Long, straight hyphae ran the entire length of the petals (figure 3), and haustoria were extended into many of the cells. The mycelium was traceable from the receptacle into the stamens, where it could be followed through the filament into the anthers (figure 4). All parts of the anthers were invaded, with the exception of the pollen cavity. The mycelium was especially abundant in all parts of the ovary.

The most significant fact in relation to the mycelial invasion of the

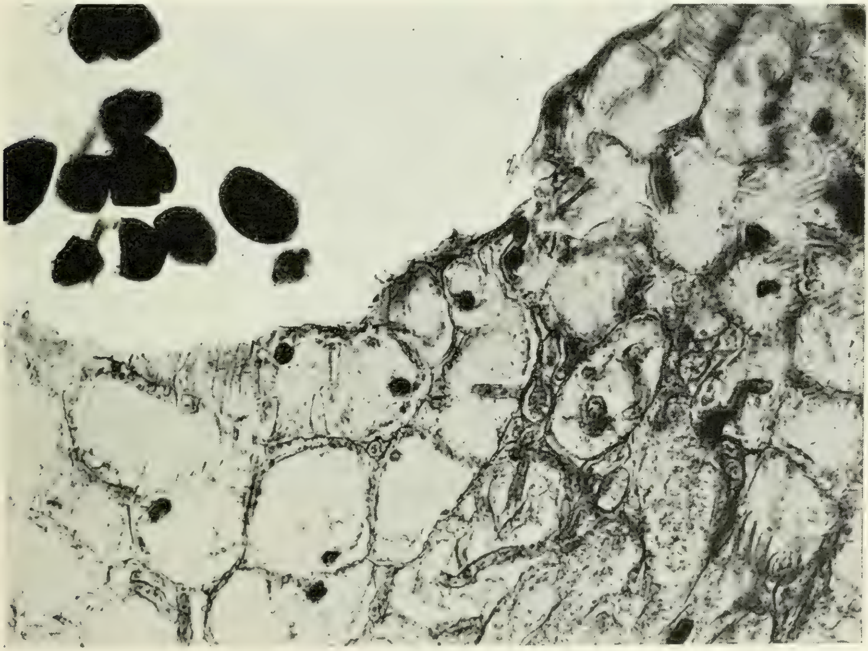

FIGURE 4. SECTION OF AN ANTHER OF AN INFECTED ONION FLOWER, SHOWING THE PRESENCE OF THE MYCELIUM OF THE FUNGUS

flower is that the fungus was present in the ovule (figures 5 and 6). A dense mat of mycelium was formed in the base of this organ (figure 6), and also was distributed entirely around the ovule. Since the only material found was immature flowers, it was impossible to determine which tissues of the mature seed would contain the fungus. Nevertheless the presence of the mycelium in such a large quantity in the base of the ovule suggests that, even though the embryo might not be invaded, the fungus would remain in the seed coat and the seedling would become infected during germination. As a rule, the seed coat of the onion remains attached to the cotyledon.

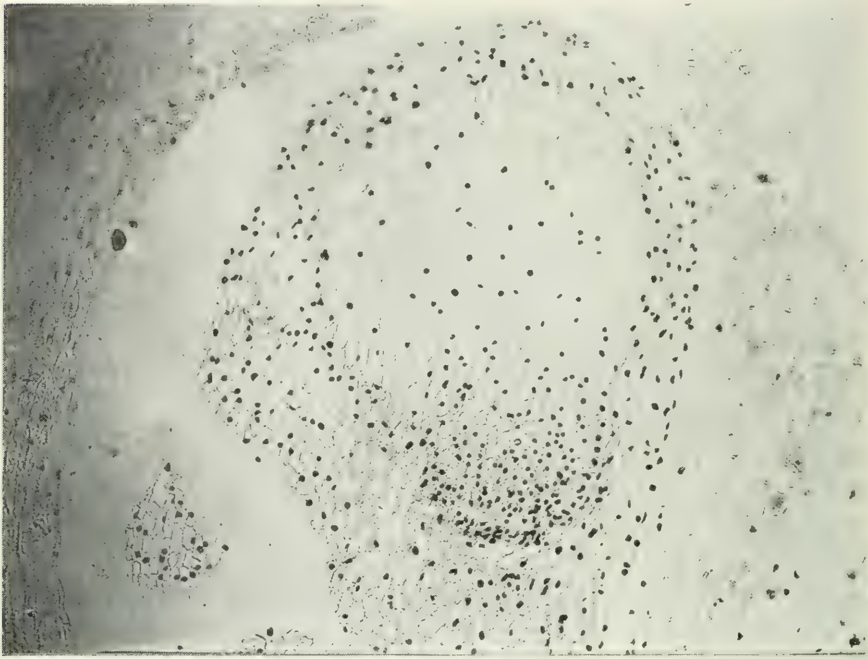

FIGURE 5. LONGITUDINAL SECTION SHOWING PART OF THE OVARY AND ONE OVULE OF AN INFECTED ONION FLOWER

Katterfeld (1926), working in Russia, found that the mycelium almost reached the base of the ovary, but he could find none in the seed. Hiura (1930 a), working in Japan, found that the tissues of the flower stalks, and the perianths, styles, ovaries, filaments, and anthers of the flowers, from diseased shoots contained abundant hyphae. He did not report the presence of the fungus in the ovules, however.

Katterfeld (1926), Murphy and M'Kay (1926), and Hiura (1930 a) obtained negative results in experiments on seed transmission. In the two latter investigations, seed from infected plants were planted but none of the seedlings developed the disease; while Katterfeld made a microscopic examination of the seed from diseased plants and was unable to find the fungus. The results obtained by these investigators are not surprising, since it is probable that only a small proportion of seed from diseased plants is infected. Although many of the seedstalks may bear several mildew lesions, it is only rarely that the fungus is found on the flower pedicels. Even when it is found fruiting on some of the flower pedicels, usually others in the same umbel are not invaded. It is probable also that only a small number of the invaded flowers mature viable seed.

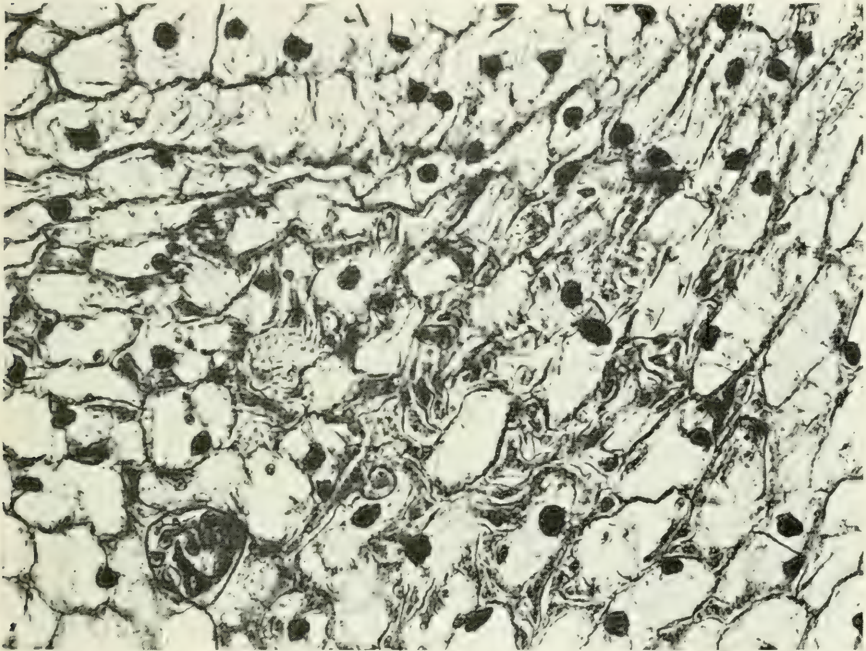

Figure 6. longitudinal section of an ovule of an infected onion flower, showing a mat of mycelium in the tissues at the base

Since obtaining the above evidence that *Peronospora destructor* may be transmitted in the seed, a review of the literature and further work with other downy mildews has shown that seed transmission of members of the Peronosporales is probably of frequent occurrence and is one of the principal means by which they overwinter and are disseminated. Although seed transmission of *Phytophthora phaseoli* has not been actually demonstrated, the work of Clinton (1906) has shown that it probably does occur. Angell (1929) gives relatively conclusive experimental evidence that the organism causing blue mold of tobacco is carried in or with the seed. The manner in which this disease made its first appearance in this country would indicate also that the inoculum was widely distributed and needed only the proper environmental conditions for its development. Wolf and Lehman (1924), in their studies on downy mildew of soybean, found circumstantial evidence that *Peronospora manshurica* is transmitted with the seed. Leach (1931) found mycelium and oospores of *Peronospora schachtii* in the sepals, pericarp, filaments, and ovules of beet flowers, but was unable to find them in the nucellus and the embryo. By controlled germination tests, however, he was able to obtain evidence of seed transmission of the beet-downy-mildew fungus. According to the *Plant Disease Reporter,* beet mildew is as severe on beet seed-plants in California as is onion mildew on onion seed-plants. Eriksson (1925) also cites evidence of seed transmission of beet mildew. The writer's own observations on the downy mildews of spinach, crucifers, and cucurbits point to seed transmission of the causal organisms of these diseases. It has been observed frequently that cucumbers are attacked by mildew as early and as severely when grown on new and isolated fields as when grown on old land or near other fields of cucumbers. *Peronospora parasitica* frequently was found fruiting on the seed pods of kale and cabbage, and it is probable that the fungus enters some of the seed. Observations have shown that *Peronospora effusa* is present on practically all spinach seed-plants, and that it fruits on some of the seed. Sections of these seed have demonstrated the presence of the fungus in the ovary and in the seed coat. The seed of commerce consists of the ovule surrounded by the ovary.

ENVIRONMENTAL FACTORS

FIELD OBSERVATIONS

In studying onion mildew in the field, an attempt was made to determine the climatic conditions favoring the development and spread of the disease. However, with so many variable factors only general conclusions could be reached. It was observed that, as a rule, mildew was not found until late July or early August, when the nights were relatively cool and heavy dews occurred frequently. Prolonged rainy and muggy periods also were very favorable for the development and spread of the disease. The fungus was found capable of producing conidiophores and conidia at rather low temperatures. The fresh fruiting structures were found on numerous occasions at Elba during the latter part of September, when the night temperature was as low as 5° C. Conidia were found also on Egyptian onions as late as the middle of November in 1929 at Ithaca.

EXPERIMENTAL WORK

Relation of temperature to the formation of conidiophores and conidia

Experiments on the relation of temperature to the formation of conidia were not conducted in carefully controlled chambers. However, a few tests were made on this phase of the problem in the greenhouse and the laboratory. The results of these tests are presented here, since they show the approximate temperature range over which conidia may be formed.

Infected plants were placed at four different temperatures in the greenhouse. One test was sufficient to show that the fungus would fruit at all temperatures tested (10° to 28° C.). Following this test, two infected plants were brought into the laboratory, sprayed with water, and treated as follows: one was placed in a 3°-C. incubator under a bell glass to prevent evaporation of the water, while the other was placed under a bell glass in a room where the temperature rose as high as 34° C. during the night. The next morning fruiting structures were found on both plants. There is no question about the conidia having been formed at 3° C., because the temperature remained constant in the incubator. It is possible, however, that 34° C. is too high a temperature for the formation of conidia, and that in this case they were formed before the temperature rose to that degree. Murphy and M'Kay (1926) found that conidiophores were first produced when the maximum temperature was 10° C., but that they were much more abundant several days later, when it rose to 23°. Katterfeld (1926) found that they were formed at 9.5° to 11° C. These workers did not report the determination of the full range of temperature over which conidiophores are formed. Hiura (1930 b) found that the optimum temperature for the production of conidia was near 15° C., the minimum was 6°, and the maximum was 25°.

Relation of humidity to the formation of conidiophores and conidia

As stated above, preliminary observations and experiments in the field and the greenhouse indicated that an abundance of moisture is necessary for the formation of conidiophores. In order to determine definitely the hygroscopic requirements of the fungus for fruiting, a series of experiments was run at the New York State Agricultural Experiment Station at Geneva, where a chamber in which the humidity could be controlled was made available through the kindness of Dr. J. G. Horsfall. The plants used in these experiments were inoculated in the greenhouse at Ithaca and were taken to Geneva after the period of incubation had passed. Accident reduced the number of plants that could be used successfully in the first experiments, but the test was repeated later for the writer by Dr. Horsfall with a different set of inoculated plants.

The experiments were conducted at night, since humidities could not be maintained in the chambers under conditions existing during the day.

The following plan of experiment was adopted. As many plants as the chamber would hold were thoroughly watered and placed in the chamber late in the afternoon. Some of these were atomized with water and covered with bell glasses to make sure that the water would remain on the leaves all night. The hydrostat was set for as near 100 per cent humidity as was possible. A hygrothermograph placed in a box gave a complete record of the temperature and humidity, and a sling psychrometer placed where it would be exposed to the current of air set up by a fan furnished an additional check on the temperature and humidity.

The plants under the bell glasses were in an atmosphere of 100 per cent humidity with water on the leaves, while the others were in an atmosphere of slightly lower humidity and without visible water on the leaves. Plants that did not produce conidia under the conditions of the experiments were afterward tested to make sure that they were infected. This was accomplished by spraying the plants with water and placing them under bell glasses on the greenhouse bench or in a chamber with high humidity. Only those that were proved to have been infected are included in the experimental results.

The plan of the second experiment was changed on February 21. On that date the plants under the bell glasses were not watered, and so their leaves remained dry, while those in the humidity chamber were sprayed continually with water.

The results of the experiment on humidity are shown in table 3. An examination of this table shows that twenty-one of the twenty-two plants produced conidia when they were exposed to high humidities (98 to 100 per cent) and when there was visible water present on the leaves; thirteen of these twenty-one had previously failed to produce conidia when exposed to high humidities but without water on their leaves. The results of these tests indicate that water must actually be present on the leaves for conidiophores and conidia to be formed. Two of the twenty-two plants formed conidiophores and conidia when

TABLE 3. Relation of Humidity to the Formation of Conidiophores and Conidia

Plant no.	Date	Treatment	Relative humidity (per cent)	Leaves wet or dry	Conidia present or absent
First series of tests					
1	Dec. 1	Humidity chamber	100	Wet	+
8	Dec. 1	Humidity chamber	100	Wet	+
25	Dec. 1	Humidity chamber	100	Wet	+
55	Dec. 5	Humidity chamber	99	Dry	−
55	Dec. 6	Inoculation chamber	100	Wet	+
66	Dec. 4	Humidity chamber (B. G.)*	100	Wet	+
67	Dec. 6	Humidity chamber	98	Dry	−
67	Dec. 7	Inoculation chamber	100	Wet	+
69	Dec. 4	Humidity chamber	98	Dry	−
69	Dec. 5	Bell glass on bench	100	Wet	+
51	Dec. 6	Humidity chamber	99	Dry	−
51	Dec. 7	Inoculation chamber	100	Wet	+
34	Dec. 6	Humidity chamber (B. G.)	100	Dry	−
34	Dec. 7	Inoculation chamber	100	Wet	+
40	Dec. 6	Humidity chamber	99	Dry	−
40	Dec. 7	Inoculation chamber	100	Wet	+
81	Dec. 4	Humidity chamber	98	Dry	−
81	Dec. 7	Inoculation chamber	100	Wet	+
73	Dec. 4	Humidity chamber	98	Dry	−
73	Dec. 7	Inoculation chamber	100	Wet	+
50	Dec. 6	Humidity chamber	99	Dry	−
50	Dec. 7	Inoculation chamber	100	Wet	+
Second series of tests					
10	Feb. 19	Humidity chamber	99	Dry	−
10	Feb. 21	Humidity chamber (B. G.)	100	Dry	+
12	Feb. 19	Humidity chamber	99	Dry	−
12	Feb. 21	Humidity chamber	100	Wet	+
23	Feb. 19	Humidity chamber	99	Dry	−
23	Feb. 21	Humidity chamber	100	Wet	+
29	Feb. 19	Humidity chamber	99	Dry	−
29	Feb. 20	Humidity chamber (B. G.)	100	Wet	+
29	Feb. 21	Humidity chamber	100	Wet	+
30	Feb. 19	Humidity chamber	99	Dry	−
30	Feb. 20	Humidity chamber (B. G.)	100	Wet	+
75	Feb. 19	Humidity chamber (B. G.)	100	Wet	+
75	Feb. 21	Humidity chamber (B. G.)	100	Dry	+
81	Feb. 19	Humidity chamber (B. G.)	100	Wet	+
81	Feb. 20	Humidity chamber (B. G.)	100	Wet	+
81	Feb. 21	Humidity chamber (B. G.)	100	Wet	+
83	Feb. 19	Humidity chamber	100	Wet	+
89	Feb. 19	Humidity chamber (B. G.)	100	Wet	+
89	Feb. 21	Humidity chamber	100	Wet	+
92	Feb. 20	Humidity chamber (B. G.)	100	Wet	+
92	Feb. 21	Humidity chamber (B. G.)	100	Wet	+

* "B. G.", plants covered with a bell glass.

exposed to high humidities without water on their leaves. This was probably because sufficient water had accumulated at the stomata during transpiration in the humid atmosphere to allow the formation of the fruiting structures. It is possible that conidia would always be formed in an atmosphere of 100 per cent humidity, provided that sufficient time were allowed for water to accumulate around the stomata.

These tests supported the conclusions reached from field observations and greenhouse practices. In the field it was always noted that the fungus fruited only when water was present on the leaves in the form of rain or dew, and in the greenhouse the diseased plants were always sprayed with water and covered with a bell glass to obtain conidia. On a number of occasions a few conidia were formed on plants in the greenhouse which had not been sprayed with water and covered with bell glasses. This occurred only at times when the humidity was very high, and water had probably accumulated around the stomata during transpiration as occurred in two of the plants included in table 3.

SPORE GERMINATION

Preliminary studies on spore germination made at the field laboratory indicated that in order to obtain good germination it was necessary to control the environmental factors as well as to have fresh spores. The introduction of the fungus into the greenhouse at Cornell University in the fall of 1929 made possible further studies on germination.

TECHNIC

Fresh spores for use in these tests were obtained by spraying an infected plant in the greenhouse with water and placing it under a bell glass for the night. On the following morning the fresh conidia that had been formed during the night were brought into suspension by dipping the mildewed leaves into water. In this manner only fresh mature spores were obtained, and they were protected from desiccation since they were not exposed for any length of time to the air. Drops of the suspension were placed on clean microscope slides in moist chambers and these were placed in incubators at different temperatures. The necessary apparatus for the tests was placed in the incubators the night before, so that it would be at the temperature at which the spores were to be tested.

Distilled water was used at first as a suspension medium for the spores, but, since many of the spores were ruptured owing to high osmotic pressure, it was necessary to find a more favorable medium. A test in which the germination of the spores in distilled water, in lake water, and in physiological salt solution, was compared, showed lake water to be a suitable medium. The results of the test are given in table 4.

This test showed that lake water and physiological salt solution were more favorable media than distilled water for spore-germination tests because they were more nearly isotonic with the spore contents. Since the best germination was obtained in lake water, this was used in all the subsequent preparations of spore suspensions. In all cases the water was collected in Erlenmeyer flasks, and was sterilized before being used in order to prevent the development of bacteria and algae.

TABLE 4. EFFECT OF SUSPENSION MEDIUM ON SPORE GERMINATION

Suspension medium	At 12° C.		At 15° C.	
	Per cent germinated	Per cent ruptured	Per cent germinated	Per cent ruptured
Distilled water	46	38	18	51
Lake water	75	4	74	7
Physiological salt solution *	65	0	31	2

* The physiological salt solution was an 0.85-per-cent solution of sodium chloride in distilled water.

EFFECT OF TEMPERATURE ON THE TIME AND THE PERCENTAGE OF GERMINATION OF THE CONIDIA

After the above preliminary tests had been completed, germination experiments were conducted at the following temperatures: 3°, 6°, 11°, 13°, 18°, 21°, 27°, 30°, and 35° C. The spores used in these tests were prepared as described above. Six slides, each slide bearing three drops of spore suspension, were used for each temperature. One slide for

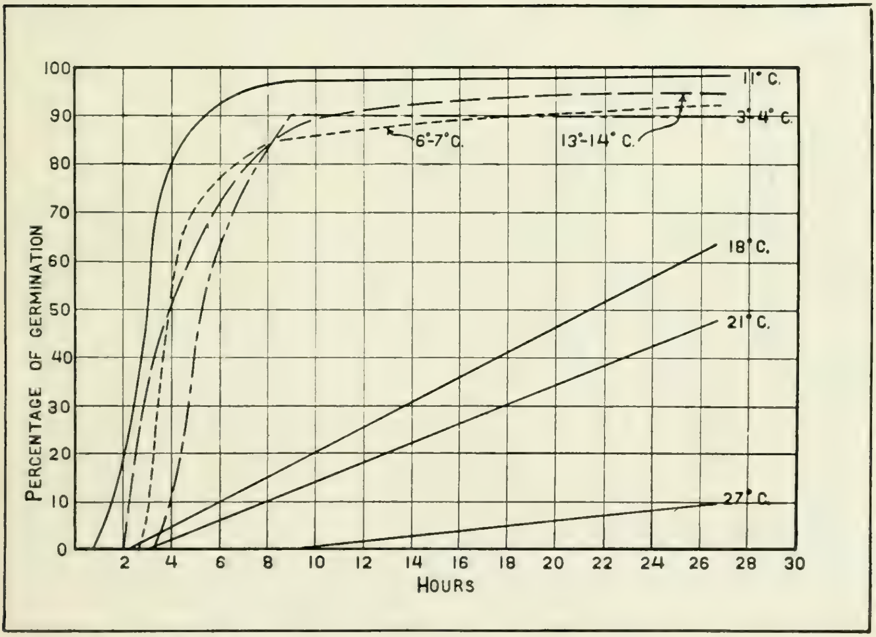

FIGURE 7. EFFECT OF TEMPERATURE ON THE TIME AND THE PERCENTAGE OF GERMINATION OF CONIDIA OF PERONOSPORA DESTRUCTOR

each temperature was examined at the end of two, three, four, five, seven, and twenty-four hours, respectively, and the percentage of germination in each drop was noted. The percentage of germination was obtained by counting 100 spores in each drop. The results, presented in figure 7, are based on three replications and on counts of approximately 5500 spores for each temperature tested.

According to these results the optimum temperature for germination of these spores is 11° C., since at this temperature germination was the most rapid and complete. Within six hours approximately all of the spores had germinated. Spores kept at lower temperatures germinated slightly more slowly, but the final percentage of germination was nearly as high. Those kept at 13° to 14° behaved similarly. At 18° and above, the higher the temperature, the lower was the final percentage of germination. No germination occurred above 27°.

Temperature also had an effect on the type of germ tube formed.

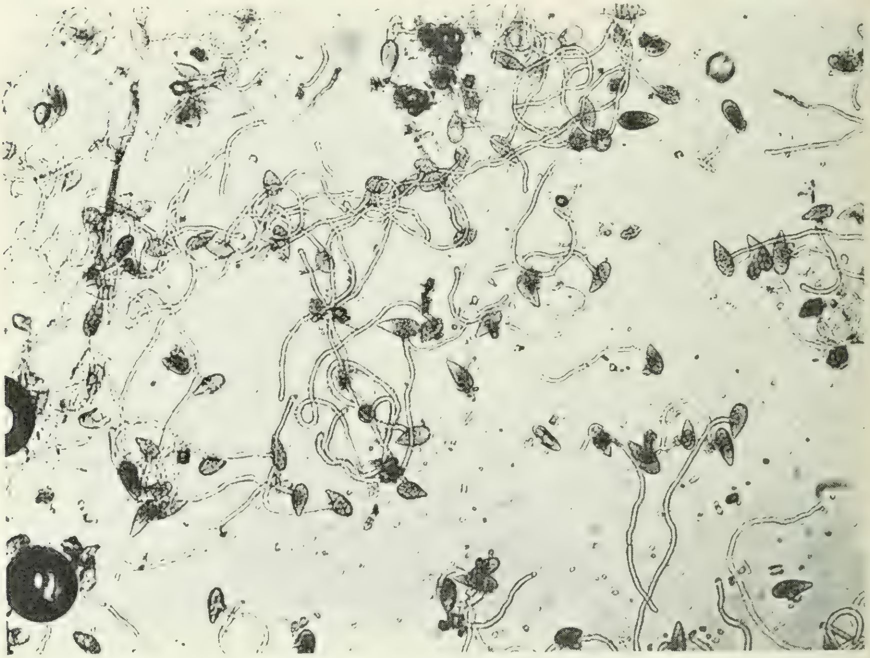

FIGURE 8. GERMINATION OF CONIDIA OF PERONOSPORA DESTRUCTOR AT THE OPTIMUM TEMPERATURE

Germ tubes developed at or near the optimum temperature for germination were the longest and appeared to be the most nearly normal in shape (figure 8). Those produced at the high temperatures were short and tended to be misshapen (figure 9), while those at the low temperatures were longer than those at the high temperatures but shorter than those at the optimum. The peculiar type of germ tube produced at low temperatures is illustrated in figure 10.

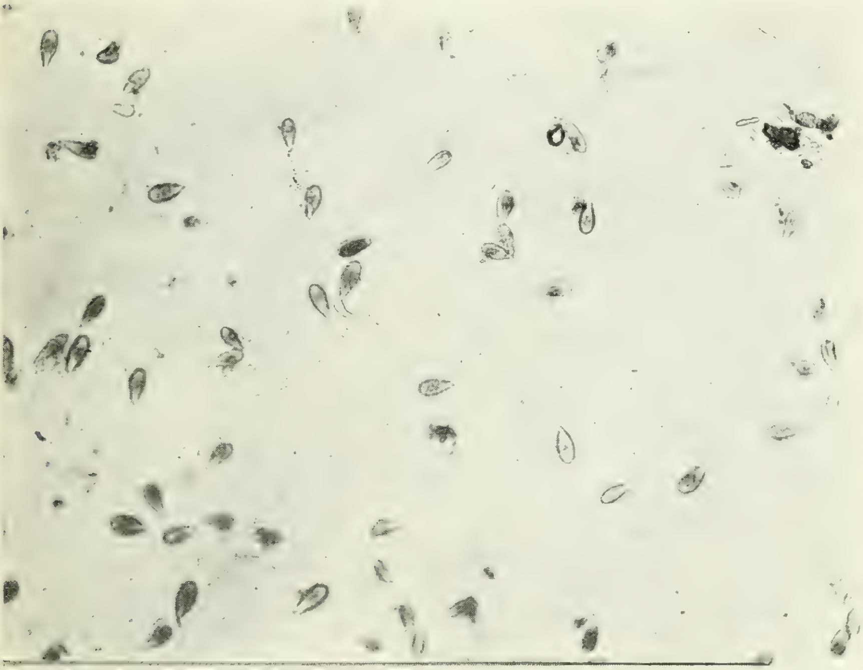

FIGURE 9. GERMINATION OF CONIDIA OF PERONOSPORA DESTRUCTOR AT HIGH TEMPERATURES

Normal germination is by a single germ tube, or occasionally two tubes, produced from the side of the conidium. Less frequently, a single germ tube may be produced from the apical end of the spore. The germ tubes are uniform in diameter, measuring from 5.76 to 7.68 microns, and have been observed to attain a length of 940 microns. As a rule they remain unbranched, but occasionally they undergo branching. The latter condition is found at times in rain water, in

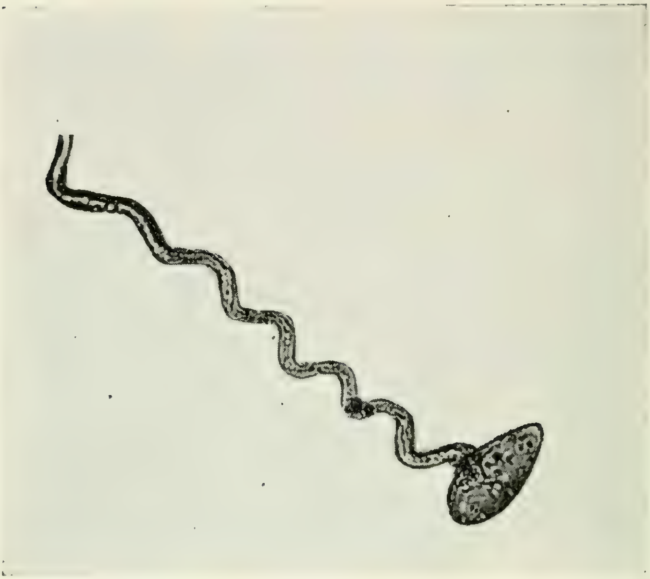

FIGURE 10. GERMINATION OF CONIDIA OF PERONOSPORA DESTRUCTOR AT LOW TEMPERATURES

dew collected from onion leaves, and in Knop's nutrient solution (figure 11).

Murphy and M'Kay (1926) obtained germination at 8.5 to 10° C., a trace at 25°, and none at 30°. They do not report temperatures lower than 8.5° C., nor apparently did they determine the exact optimum temperature. Katterfeld (1926) studied the germination in considerable detail, paying especial attention to the rate of growth of the germ tubes, the rate of germination, and the ability of the conidia to germinate. His tests were run at a laboratory temperature of 10° to 12° R. (12.5° to 15° C.) and 14° R. (17.5° C.), which, according to the writer's experiments, are favorable temperatures for germination although a little higher than the optimum. By making observations every one and one-half hours Katterfeld found that at a temperature of 15° C. the rate of growth of the germ tubes during the first five and one-half hours varied from 46 to 111 microns and averaged 101 microns per hour. Although the writer did not make a special study of the rate of growth, his observations in general confirm Katterfeld's in that the

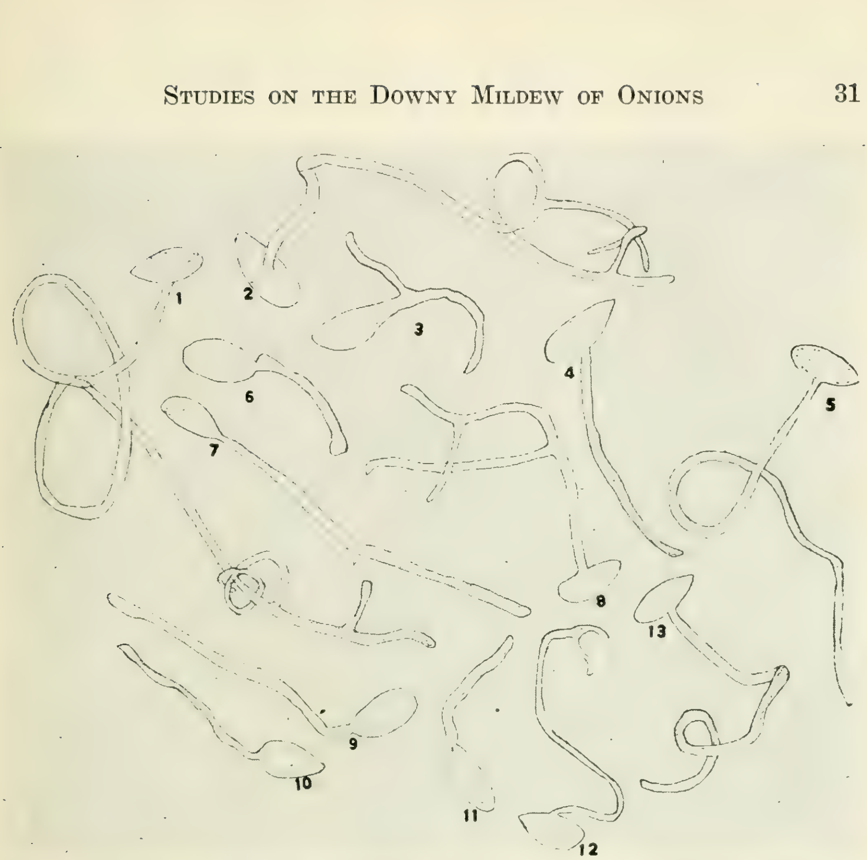

FIGURE 11. VARIOUS TYPES OF GERMINATION OF THE CONIDIA OF PERONOSPORA DESTRUCTOR

germ tubes were found to grow very rapidly and the growth rate increased with the increase in temperature up to about 21° C. In studying the rate of germination, Katterfeld found that, where he obtained a germination of 100 per cent, from 64 to 100 per cent of the spores germinated between two and two and one-half hours after being sown, and that the rate was lower when the total germination was lower. He found that all viable conidia germinated in five hours. These results differ from those presented in figure 7, in that at the optimum temperature only 50 to 60 per cent of the conidia had germinated by the end of the first two and one-half hours and complete germination was not accomplished for about twenty-four hours.

Katterfeld found that conidia capable of germination could be readily recognized by their appearance. The viable conidia refracted light, had tense walls, and were completely filled with protoplasm. Loss of vitality was shown by a loss of tension and a withdrawal of the cytoplasm from the walls. Katterfeld found that immature conidia were capable of germination but produced shorter germ tubes than did mature conidia. Conidia exposed to an atmosphere of 100 per cent humidity lost their germinating power after fifteen to seventeen hours, while those exposed to dry laboratory air were not able to germinate after one and one-half to two hours. The writer is in agreement with Katterfeld on these points.

Hiura (1930 b) found that the time required for the germination of conidia varies with the temperature and with the conidia. His conclusions in regard to the optimum, minimum, and maximum temperatures for conidial germination are approximately the same as those of the writer. He found the optimum to be near 10° C., the maximum near 20°, and the minimum below 5°. His results regarding the time required for germination do not agree wholly with those obtained in this study, however. According to Hiura's results, the time required for germination decreases up to the optimum temperature, but does not increase appreciably above the optimum as was shown by the writer's results.

INFECTION

Infection is dependent on an abundance of viable conidia and environmental conditions favoring their rapid germination. In practice it was found that by applying the information obtained in the experiments on the relation of temperature and moisture to spore formation and germination, infection could always be obtained. Infection was determined by the production of conidia when the plants were placed under favorable conditions for their development.

Spores were obtained for inoculation as described under *Technic* (page 26), and were immediately taken to the laboratory. Here potted plants were sprayed thoroughly with the spore suspension and were then placed under moist bell glasses in the incubators at temperatures below 18° C. for twelve to twenty-four hours. The plants were then removed to the greenhouse, where they were kept in a cool room with a maximum temperature of approximately 20° C. and a minimum temperature of about 10°. Plants inoculated in this manner always became infected; but infection seldom took place when the inoculum had been exposed to dry air or to high temperatures, or when the plants were not kept cool for at least twelve hours after inoculation.

INCUBATION PERIOD

The incubation period was determined by inoculating a group of potted plants under favorable conditions, as described above, and then testing for infection by placing moist bell glasses over the plants. In order to avoid injury by Botrytis, the plants were divided into two groups and these groups were tested on alternate days by covering with bell glasses. In this manner neither group was exposed continuously to a humid atmosphere. The temperature of the greenhouse in which these tests were conducted was from 10° to 24° C. The results obtained in two of these tests are shown in table 5.

TABLE 5. INCUBATION PERIOD OF PERONOSPORA DESTRUCTOR IN ALLIUM CEPA

Number of plants	Number of days between inoculation and the production of conidia
3	11
3	12
2	13
2	14
1	15

From these results it is seen that the incubation period is from eleven to fifteen days under the conditions of this experiment. Of twenty-three other plants that were placed under bell glasses between thirteen and twenty days after inoculation, twenty-two produced conidia, indicating that the incubation period had already been completed. These results agree with those of Katterfeld (1926), who found the incubation period to be ten to fifteen days in the greenhouse and thirteen to eighteen days in the garden. They differ from those of Murphy and M'Kay (1926), who found the period to be twenty-three days.

EFFECT OF TEMPERATURE ON INFECTION

Eight plants were inoculated under favorable conditions for infection on January 25, and on the following day they were transferred to the greenhouse, where half of them were placed in a warm room (19° to 33° C.) and the other half in a cool room (10° to 24° C.). Beginning six days after inoculation, half of the plants in each house were tested for conidial production every other day by placing them under bell glasses after spraying them with water. The results of these tests are shown in table 6.

This experiment indicated that high temperatures are unfavorable for the development of the fungus in the host tissues. Even though all of the plants were inoculated and treated alike during the first twenty-four hours, none of those kept at the warm temperature developed conidia although all four of those kept in the cool house produced them in eleven to sixteen days after inoculation. The tests

TABLE 6. EFFECT OF TEMPERATURE ON INFECTION OF ONIONS BY PERONOSPORA DESTRUCTOR*

Number of days after inoculation	Warm house				Cool house			
	Plant no.				Plant no.			
	94	95	96	97	98	99	100	101
6	.....	.....	.....	.....	.....	.....	.....	......
7	–	–	.....	.....	–	–	.....	......
8	.....	.....	–	–	.....	.....	–	–
9	.....	.....	.....	.....	.....	.....	.....	......
10	–	–	.....	.....	–	–	.....	......
11	.....	.....	–	–	.....	.....	–	+
12	–	–	.....	.....	–	+	.....	......
13	.....	.....	–	–	.....	.....	+	+
14	–	–	.....	.....	+	+	.....	......
15	.....	.....	.....	.....	.....	.....	.....	+
16 †	.....	–	.....	.....	+	.....	.....	......

* The minus and plus signs in the body of the table indicate the absence or the presence of conidial formation following a test for infection.

† The experiment was continued through twenty-nine days without further results.

were discontinued after the twenty-ninth day, since by that time none of the plants in the warm house had produced conidia. It is possible that the fungus may have been killed by the high temperature in the warm house.

CONTROL

The field application of fungicides which was formerly recommended for the control of onion mildew was found during the investigation to be unpractical, costly, and of doubtful value. It is unpractical to spray or dust with the usual mixtures now available, because the waxy covering of the leaves prevents the materials from adhering; and the thick top growth makes it very difficult, at least, to spray or dust without causing serious injury to the plants. In order to protect the plant properly, it would be necessary to begin spraying or dusting in the seedling stage and to continue the treatment until shortly before harvest. This would make the cost of growing onions prohibitive. Spraying with 4–4–50 bordeaux mixture and dusting with 20–80 copper-lime dust and Kolodust, in the course of this study, failed to give any indication of controlling the disease.

A more practical and beneficial means of control is considered to be the exclusion of the fungus from new land or from fields that have not been used for onions for several years. Sanitation, rotation, and avoidance of the environmental conditions favorable for the spread of mildew, are additional measures to be recommended.

It may be possible to accomplish the first of these measures by using only disease-free seed and sets, and by avoiding the introduction of soil from infested fields on tools and on transplants. It is possible that disease-free seed may be procured from sections of the country in which onion mildew does not occur, or perhaps it can be grown from disease-free seed bulbs. Such bulbs may be obtained by selection or by treating with hot air at 40° C. for eight hours. Murphy and M'Kay (1926) found that this treatment would kill the fungus in the bulb. Disease-free sets also may be obtained in this manner.

Sanitation and rotation will aid in reducing the amount of inoculum in the soil. The onion refuse consists largely of tops which may contain oospores and of culls which may be systemically infected. Practically 100 per cent of the onion refuse was burned at Elba in the fall of 1927, 1928, and 1929 for the control of onion maggots. This practice was accompanied by a decrease in the severity of onion mildew during those years. At present the length of time that the fungus will remain viable in the soil is not known, and so the length of rotation necessary for the elimination of the fungus from the soil is uncertain.

The severity of the disease can be reduced to some extent by regulating the environmental conditions. The location of the onion field has much to do with prevalence of the disease. As a rule, onions grown in fields that are well drained and are exposed to the sun and wind suffer less from mildew than do those in fields on low ground or surrounded by high windbreaks. Furthermore, no work should be done in the onion fields while the leaves are wet with dew or rain, because the spores are viable under such conditions and may be carried on the clothing of the workmen to other plants in the field.

SUMMARY

Downy mildew, caused by *Peronospora destructor* (Berk.) Caspary, is one of the most serious diseases of the onion crop.

Evidence has been presented to show that *Peronospora destructor* (Berk.) Caspary is the valid name of the causal fungus.

The disease is widely distributed, occurring in nearly all parts of the world and in most of the principal onion-growing sections of the United States.

A brief history and review of the literature of onion mildew is given.

Peronospora destructor has previously been recorded on seven species and varieties of Allium. *Allium schoenoprasum* is here recorded as a new suscept. Fifty-three varieties of the common onion were tested and found to be equally susceptible to onion mildew.

Losses may be sustained at any stage in the life of the plant. Data are presented to show that the bulbs are considerably stunted by the disease.

Field observations were made on the occurrence of the disease on market onions, set onions, seed onions, and Egyptian onions. The disease was found to be favored by abundant moisture and relatively low temperatures. Market onions were found to become diseased as early and as frequently on new as on old land. The first diseased plants were found to be scattered throughout the fields.

There are four sources of primary inoculum: systemically infected plants, infested soil, infested seed, and infected seed. Evidence presented points to the fact that mycelium in the seed is an important source of primary inoculum.

The fungus was found to fruit over a wide range of temperatures. Water on the leaves was necessary for the formation of conidiophores and conidia.

Lake water was found to be the most satisfactory medium tested for spore germination. The optimum temperature for spore germination was found to be 11° C., but the full range over which the spores germinate was 3° to 27° C.

An abundance of moisture, and low temperatures, were found necessary for infection. The incubation period was about eleven to fifteen days.

Fungicidal treatment is not considered a satisfactory method of control. Exclusion of the fungus from new areas, sanitation, and regulation of the environmental conditions, are considered the most promising means of combating the disease.

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